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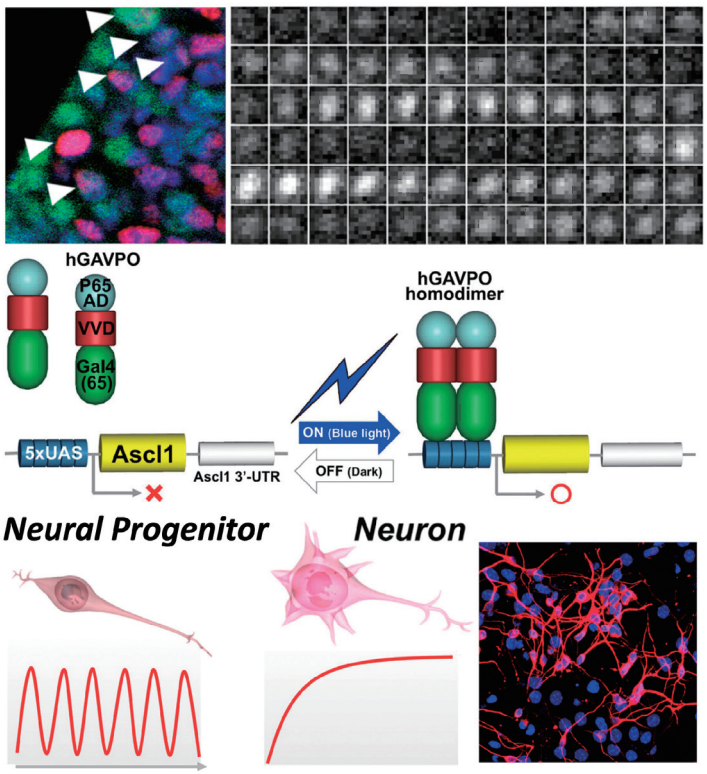

京都大学ウイルス研究所年報

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CHRONOLOGICAL TABLE

1956 April	Institute for Virus Research, Kyoto University, was founded with two departments (Pathology and Biophysics).
1956 April	Scientific Lectures for the Public were presented commemorating the opening of the Institute (the successive Memorial Lecture Series have been presented annually hereafter).
1957 April	Department of Biochemistry and Department of Serology and Immunology were established.
1958 April	Department of Prevention and Therapeutics was established.
1958 December	"Advances in Virology", Vol. 1 (in Japanese) was published as collection of the Memorial Lectures (the successive volumes were published annually hereafter until 1960).
1958 December	"Annual Report of the Institute for Virus Research", Vol. 1, was published (the successive volumes have been published annually hereafter).
1959 July	Virus Diagnosis Center was established.
1961 October	The 1st Symposium of the Institute for Virus Research was held under the auspices of the Institute with the nationwide participants. The proceedings of the Symposium were published as the first issue of the new series of "Advances in Virology" in Japanese (the successive Symposia have been held and their proceedings published annually hereafter).
1962 April	Department of Tumor Virus was established.
1962 October	Several staff members were appointed academic staff of the Graduate School of Medicine, and students of the School were first admitted to the Institute.
1962 December	Several staff members were appointed academic staff of the Graduate School of Science, and students of the School were first admitted to the Institute.
1964 April	Virus Diagnosis Center was renamed Virological Diagnosis Center.
1965 September	Construction of the new building for the Institute commenced.
1967 March	Construction of the new building was completed.
1968 April	Department of Genetics was established.
1974 April	Department of Molecular and Cellular Virology was established.
1977 April	Department of Neurological Virus Disease was established as such that Visiting Staff be appointed.
1978 April	Animal Laboratory for Experimental Virus Infection was established.
1981 March	Construction of extension of the main building was completed. Thus the main building now constitutes five floors with a basement occupying the aggregate area of 5,410 ㎡. The major part (ca. 481 ㎡) of the extended area serves for researches

	involving radioisotope labelling and in vitro DNA recombination experiments requiring the P3 facilities.
1986 May	The memorial events for the 30th anniversary of foundation of this Institute were held on May 16-17.
1986 November	Professor Yorio Hinuma was honoured as "Person of Cultural Merits (Bunkakorosha)"
1987 May	Department of Biophysics and Department of Tumor Virology were reorganized to form Department of Viral Oncology which consists of 4 Laboratories.
1988 April	Virological Diagnosis Center was reorganized to become Research Center for Immunodeficiency Virus which consists of Laboratory for AIDS Immunology and Laboratory of Viral Pathogenesis.
1989 April	Department of Biochemistry and Department of Genetics were reorganized to form Department of Genetics and Molecular Biology which consists of 3 Laboratories.
1990 March	Construction of a new building was partly completed.
1990 April	Department of Pathology and Department of Molecular and Cellular Virology were reorganized to form Department of Cell Biology which consists of 3 Laboratories, while Department of Serology and Immunology, Department of Prevention and Therapeutics and Department of Neurological Virus Disease were reorganized to form Department of Biological Responses which consists of 2 laboratories and one for visiting staff.
1992 April	Laboratory of Regulatory Information was established within the Department of Cell Biology to host a visiting professor as well as a research group.
1993 December	Construction of the new building which accommodates three laboratories from this Institute as well as some from the Medical School and the Center for Molecular Biology and Genetics of the University was completed.
1994 October	Construction of a new animal facility with some laboratories was completed.
1998 April	One stuff member was appointed academic staff of the Graduate School of Pharmaceutical Sciences, and students of the school were first admitted to the Institute.
1998 April	Research Center for Immunodeficiency Virus was reorganized to become Research Center for Acquired Immunodeficiency Syndrome.
1998 April	Laboratory of Virus Control in Research Center for Immunodeficiency Virus was established as such that Visiting Stuff be appointed.
1999 April	Several staff members were appointed academic staff of the Graduate School of Biostudies, and students of the school were first admitted to the Institute.
2002 April	The Experimental Research Center for Infected Animals was abolished and the Experimental Research Center for Infectious Diseases was established instead.

2005 April	Research Center for Emerging Virus was established.
2009 June	The Institute commenced service as a Joint Usage / Research Center for fusion of advanced technologies and innovative approaches to viral infections and life science.
2010 April	Center for Acquired Immunodeficiency Syndrome Research was reorganized to become Center for Human Retrovirus Research.
2010 April	Research Center for Emerging Virus was reorganized to become Center for Emerging Virus Research.
2013 October	Laboratory of Evolutional Virology was established in Experimental Research Center for Infectious Diseases.

ORGANIZATION AND STAFF

(as of December, 2013)

(Numerals in parentheses indicate year of association with the Institute)

Director Masao Matsuoka, M.D., D.Med.Sc.
Deputy Director Yoshio Koyanagi, M.D., D.Med.Sc.

Professors Emeriti
Yoshimi Kawade, D.Sc. (1956-1988)
Yorio Hinuma, M.D., D.Med.Sc. (1980-1988)
Masao Hanaoka, M.D., D.Med.Sc. (1959-1989)
Mutsuo Imai, D.Sc. (1965-1991)
Takashi Yura, D.Sc. (1960-1993)
Masakazu Hatanaka, M.D., D.Med.Sc. (1980-1995)
Akinori Ishimoto,M.D.,D.Med.Sc.(1964-1968, 1978-2002)
Yoshiaki Ito, M.D., D.Med.Sc. (1984-2002)
Masanori Hayami, D.V.M., D.Agr. (1988-2006)
Koreaki Ito, D.Sc. (1971-2007)
Kunitada Shimotohno, Pharm.D. (1996-2007)
Junji Yodoi, M.D., D.Med.Sc. (1989-2010)

Department of Viral Oncology

Laboratory of Gene Analysis
- Professor: Yoshinori Akiyama, D.Sc. (1988)
- Associate Professor: Hiroyuki Sakai, D.Med.Sc. (1996)
  Hiroyuki Mori, D.Sc. (1996)
- Assistant Professor: Shin-ichi Yanagawa, D.Agr. (1986)

Laboratory of Cell Regulation
- Professor: Masahiko Sugita, M.D., D.Med.Sc. (2004)
- Assistant Professor: Daisuke Morita, D.Bio. (2013)

Laboratory of Tumor Biogenesis
- Professor: Shin Yonehara, D.Sc. (1994) (concurrent)
- Assistant Professor: Akira Murakami, D.Sc. (1979)

Laboratory of Human Tumor Viruses
- Professor: Keizo Tomonaga, D.V.M., D.Vet.Med. (2011)
- Associate Professor: Makoto Hijikata, D.Med.Sc. (1997)
- Assistant Professor: Tomoyuki Honda, M.D., D.Med.Sc. (2011)

Department of Genetics and Molecular Biology

Laboratory of Molecular Genetics
- Professor: Takashi Fujita, D.Sc. (2005)
- Associate Professor: Hiroki Kato, D.Med.Sc. (2010)

Laboratory of Biochemistry
- Professor: Mutsuhito Ohno, D.Sc. (2001)

Assistant Professor	Makoto Kitabatake, D.Sc.(2004)
	Ichiro Taniguchi, D.Sc. (2007)

Department of Biological Responses

Laboratory of Biological Protection

Professor	Koichi Ikuta, M.D., D.Med.Sc. (2002)
Assistant Professor	Keiko Takemoto, D.Sc. (1992)
	Shizue Tani-ichi, D.Health Sc. (2007)
	Takahiro Hara, D. Bio. (2008)
Technical Staff	Satsuki Kitano (Konaka) (2004)

Laboratory of Infection and Prevention

Professor	Osamu Takeuchi, M.D.,Ph.D. (2012)
Associate Professor	Hiroshi Masutani, M.D., D.Med.Sc. (1992)
Assistant Professor	Takashi Mino, D.Eng. (2012)

Bioresponse Regulation Laboratory

Visiting Professor	Yoshihiro Kawaoka , D.V.M., D.Med.Sc. (2010)
Visiting Assistant Professor	Hironori Yoshiyama , D.Med.Sc. (2013)

Department of Cell Biology

Laboratory of Subcellular Biogenesis

Professor	Fumiko Toyoshima, D.Sc. (2008)
Assistant Professor	Shigeru Matsumura, D.Bio. (2008)

Laboratory of Growth Regulation

Professor	Ryoichiro Kageyama, M.D., D.Med.Sc. (1997)
Associate Professor	Toshiyuki Ohtsuka, M.D., D.Med.Sc. (2000)
Associate Professor (Hakubi)	Itaru Imayoshi, D.Bio.(2008)
Assistant Professor	Taeko Kobayashi, D.Sc. (2005)
Assistant Professor (Hakubi)	Tomoko Tateya , M.D.,D.Med.Sc.(2008)

Laboratory of Signal Transduction

Associate Professor	Takayuki Miyazawa, D.V.M., D.Vet.Med. (2005)

Laboratory of Regulatory Information

Visiting Professor	Susumu Tonegawa, Ph.D, D.Sc. (1992)

Center for Human Retrovirus Research

Laboratory of Viral Pathogenesis

Professor	Yoshio Koyanagi, M.D., D.Med.Sc. (2004)
Assistant Professor	Hirotaka Ebina, D.Med.Sc. (2009)
	Kei Sato, D.Med.Sc. (2012)

Laboratory of Virus Control

Professor	Masao Matsuoka, M.D., D.Med.Sc. (1999)
Lecturer	Jun-ichirou Yasunaga, M.D., D.Med.Sc. (2010)
Assistant Professor	Kazuya Shimura, D.Med.Sc. (2011)
Technical Staff	Junko Tanabe (2006)

Laboratory of Viral Immunology

Visiting Professor	Hiroaki Mitsuya, M.D.,Ph.D. (2012)

Experimental Research Center for Infectious Diseases

Laboratory of Mouse Model

Associate Professor	Makoto Tachibana, D.Agr. (1998)

Laboratory of Primate Model

Professor	Tatsuhiko Igarashi, D.V.M., D.Med.Sc. (2007)
Associate Professor	Tomoyuki Miura, D.V.M., D.Agr. (1988)
Assistant Professor	Takayuki Hishiki, D.Bio. (2013)

Laboratory of Virus Evolution

Professor	HirofumiAkari, D.V.M., D.Vet.Sc. (2013) (concurrent)
Associate Professor	Takayuki Miyazawa, D.V.M., D.Vet.Med. (2005)
Assistant Professor (Spe.*)	Amane Kogure, D.Med.Sc. (2013)
	Takeshi Yoshida, D.Med.Sc. (2013) (concurrent)
Technical Specialist	Hitoshi Miyachi (2006)
Technical Staff	Ai Dantsuka (2011)
	Setsuo Asahi (2013)

Center for Emerging Virus Research

Head・Professor	Yoshio Koyanagi, M.D., D.Med.Sc. (2010)
Assistant Professor (Spe.*)	Yosuke Yamaoka, Pharm.D. (2012)
	Akiko Makino, D.V.M.Ph.D. (2012)
	Yohei Hizukuri, D.Sc. (2013)

Lecturers (part time)

Makoto Miyata
Sokichi Matsumoto
Takeshi Ichinohe
Koji Yasutomo
Tamotsu Yoshimori
Shosei Yoshida
Tatsuya Saito
Yasumasa Iwatani
Hiroshi Kimura
Hirofumi Arakawa
Jiro Yasuda

Research Fellows

Eiji Ishii (Lab. of Gene Analysis)
Kan Fujino (Lab. of Human Tumor Viruses)
Yusuke Matsumoto (Lab. of Human Tumor Viruses)
Yuya Hirai (Lab. of Human Tumor Viruses)
Yuichi Abe (Lab. of Human Tumor Viruses)
Yuki Kaname (Lab. of Molecular Genetics)
Ryota Ouda (Lab. of Molecular Genetics)
Asako Mclosky (Lab. of Biochemistry)

	Tomoko Imamura (Lab. of Infection and Prevention)
	Daisuke Ori (Lab. of Infection and Prevention)
	Atsuko Wakabayashi (Lab. of Infection and Prevention)
	Akihiro Isomura (Lab. of Growth Regulation)
	Yukiko Harima (Lab. of Growth Regulation)
	Tsuyoshi Hirashima (Lab. of Growth Regulation)
	Rokusuke Yoshikawa (Lab. of Signal Transduction)
	Junpei Terakawa (Lab. of Signal Transduction)
	Matouskoba Magda (Lab. of Signal Transduction)
	Junko Takeuchi (Lab. of Viral Pathogenesis)
	Tomoko Kobayashi (Lab. of Viral Pathogenesis)
	Guangyong Ma (Lab. of Virus Control)
	Kenji Sugata (Lab. of Virus Control)

Library

Committee Chairman	Hiroyuki Mori

Administration Office

Chief Officer	Katsumi Sakamoto (2013)
General Affairs	Hiroyuki Matsunaga (2011)
	Kazue Hattori (2013)

Note
*Spe. : Program-Specific

INTERNATIONAL SYMPOSIUM

Our Institute held "*1st Kyoto International Symposium on Virus-Host Coevolution"* on November 07, 2013 at the Shiran kaikan Inamori Hall, Kyoto.

Program

Opening Remarks:

Prof. Masao Matsuoka, Institute for Virus Research, Kyoto University

Session 1: Emerging Viral Diseases and the Hosts

Dr. Takayuki Miyazawa, Institute for Virus Research, Kyoto University,
"Exogenous and Endogenous Betaretroviruses: Implications in the Evolution of Mammals"

Dr. Shigeru Morikawa , National Institute of Infectious Diseases, Japan,
"Canine Distemper Virus Crosses the Species Barrier to Macaque Monkeys"

Prof. Ayato Takada , Research Center for Zoonosis Control, Hokkaido University,
"Ecology of Avian Influenza Viruses: Topics from Surveillance in Asia and Africa"

Keynote Lectures:

Prof. Robin Weiss, Wohl Viron Centre, University College London,
"Virus-Host Coevolution and Cross-Species Infection"

Prof. Fumitoshi Ishino, Medical Research Institute, Tokyo Medical and Dental University,
"Mammalian Evolution Promoted by LTR-Retrotransposon-Derived Genes"

Session 2: Virus Infection and the Host Factors

Dr. Welkin Jonson, Biology Department, Boston College,
"Genetic Barriers to Cross-Species Transmission and Emergence of Primate Lentiviruses"

Dr. Massimo Pizzato, Centre for Integrative Biology, University of Trento, "Searching for Host Determinants of Lentivirus and Gammaretrovirus Infection"

Prof. Keizo Tomonaga, Institute for Virus Research, Kyoto University, "Endogenous Bornavirus-Like Elements: Cellular Co-option and Impact on Host Evolution"

Closing Remarks:

Prof. Hirohisa Hirai, Director, Primate Research Institute, Kyoto University

SEMINARS OF THE INSTITUTE FOR VIRUS RESEARCH

Twenty-one seminars were held at the Institute for Virus Research under the auspices of the Institute in 2013. Eleven lectures were from abroad and ten others were from Japan.

January 23 Dr. Becca Asquith, Imperial College London, UK. "What Constitutes a Protective HLA Class I Genotype in HTLV-1 Infection?"

February 7 Dr. Rob J. De Boer, Utrecht University, the Netherlands. "Analysing immune cell migration"

February 13 Dr. Naoya Ohara, Okayama University, Japan. "Mechanism of the drug-resistance of *Mycobacterium tuberculosis* against para-aminosalicylic acid (PAS) and the application"

February 26 Dr. Luis Menéndez-Arias, CSIC, Spain. "Complex mutational patterns associated with resistance to HIV-1 reverse transcriptase inhibitors"

March 22 Dr. Jun Hatakeyama, Kumamoto University, Japan. "Neuron maintains temporarily adhesion belt to regulate neuronal differentiation through Notch signaling"

March 27 Dr. Koichiro Uryu, RIKEN Advanced Science Institute, Japan. "Synchronization dynamics of migrating cells"

April 17 Dr. Keiji Hirota, Osaka University, Japan. "Function and plasticity of inflammatory T helper cells"

May 15 Dr. Hirofumi Arakawa, National Cancer Center, Japan. "Mechanism of mitochondrial quality control and Cancer ~ Novel tumor suppressor function of p53~"

May 17	Dr. Akatsuki Kimura, National Institute of Genetics, Japan. “How cell changes its interior depending on its size - a study on mitotic spindles in the *C. elegans* embryo”
June 5	Dr. Rongge Yang, WIV of CAS, China. “Molecular epidemiological study of HIV spread in China”
July 19	Dr. Makoto Miyata, Osaka City University, Japan. “Mycoplasma gliding”
July 22	Dr. Sokichi Matsumoto, Osaka City University, Japan. “Elimination of tuberculosis by investigating regulatory mechanism of dormancy and proliferation of *Mycobacterium tuberculosis”*
August 26	Dr. Daron M. Standley, Osaka University, Japan. “3D Molecular Modeling of antibodies and protein-RNA interactions”
September 9	Dr. Alexander Hoffmann, UCSD, US. “Combinatorial and Dynamical Codes to specify Immune Responses”
September 9	Dr. Dong Sung An, UCLA, US. “Developing hematopoietic stem cell based gene therapy strategies for treating HIV infected patients”
October 8	Dr. Seiichi Uchida, Kyushu University, Japan. “Bioimage-informatics – automatic quantification and knowledge discovery for bioimages”
October 28	Dr. Charles R M Bangham, Imperial College London, UK. “Clonality, latency and integration of HTLV-1 in vivo”
November 8	Dr.Tinxiao Riu, IMP, Austria. “Anatomical and functional dissection of *fruitless* positive courtship circuit”
November 18	Dr. Thirumala-Devi Kanneganti, St. Jude Children’s Research Hospital, US. “Role of NLRs in inflammation and disease”

December 17 Dr. Keith Willison, Imperial College London, UK. “Quantitative single cell and single molecule proteomics for clinical studies”

December 24 Dr. Yoshiaki Ito, National University of Singapore, “RUNX3: Is it a partner of p53 ?”

DEPARTMENT OF VIRAL ONCOLOGY
LABORATORY OF GENE ANALYSIS

I. First Group

Members

Professor	**Yoshinori Akiyama**
Associate Professor	**Hiroyuki Mori**
Assistant Professor (Spe.)	**Yohei Hizukuri (Center for Emerging Virus Research)**
Research fellow	**Eiji Ishii**
Graduate Student	**Tokuya Hattori**
	Yasushi Daimon
	Ryoji Miyazaki
	Narimasa Hasimoto
	Koichiro Akiyama
	Kazuya Mito
	Chigusa Masui
	Shinya Mizuno
	Akira Mukuno

Introduction

The research projects carried out in this group are concerned with post-translational events in the expression of genetic information. Specifically, processes of protein translation, protein translocation across and integration into the membrane, membrane protein proteolysis and extracytoplasmic stress responses are investigated by combined molecular genetic, biochemical biophysical and structural approaches.

Topics

Protease homolog BepA (YfgC) promotes assembly and degradation of β-barrel membrane proteins in *Escherichia coli*: S. NARITA[1], C. MASUI, T. SUZUKI[2], N. DOHMAE[2], and Y. AKIYAMA. ([1]University of Morioka, IVR, [2]RIKEN)

The cell envelope of gram-negative bacteria is composed of two layers of biological membranes, the outer membrane (OM) and the inner (cytoplasmic) membrane (IM). Outer

membrane proteins (OMPs) span the OM with amphipathic, antiparallel β-strands that form a barrel structure. Gram-negative bacteria are equipped with quality-control systems for the OM that sense and cope with defective biogenesis of its components. Accumulation of misfolded outer membrane proteins (OMPs) in *Escherichia coli* leads to activation of σ^E. Disruption of *bepA* (formerly *yfgC*), a σ^E-regulated gene encoding a putative periplasmic metalloprotease, sensitizes cells to multiple drugs, suggesting that it may be involved in maintaining OM integrity. However, the specific function of BepA has been unclear. We showed that BepA enhances biogenesis of LptD, an essential OMP involved in OM transport and assembly of lipopolysaccharide, by promoting rearrangement of intramolecular disulfide bonds of LptD. In addition, BepA possesses protease activity and is responsible for the degradation of incorrectly folded LptD. In the absence of periplasmic chaperone SurA, BepA also promotes degradation of BamA, the central OMP subunit of the β-barrel assembly machinery (BAM) complex. Interestingly, defective oxidative folding of LptD caused by *bepA* disruption was partially suppressed by the expression of protease-active site mutants of BepA, suggesting that BepA functions independently of its protease activity. We also showed that BepA exhibits genetic and physical interactions with components of the BAM complex. These findings raised the possibility that BepA maintains the integrity of the OM both by promoting assembly of OMPs and by proteolytically eliminating OMPs when their correct assembly is compromised[1)].

1) Narita, S.-i., *et al*. (2013) Protease homolog BepA (YfgC) promotes assembly and degradation of β-barrel membrane proteins in *Escherichia coli*. ***Proc. Natl. Acad. Sci. USA*** **110**, E3612–E3621

Membrane targeting-mediated regulation of *Escherichia coli* heat shock transcription factor, σ^{32}: B. LIM[1], R. MIYAZAKI, S. NEHER[1], D.A. SIEGELE[2], K. ITO[3], P. WALTER[1], Y. AKIYAMA, T. YURA[3], and C.A. GROSS[1] ([1]University of California, San Francisco, [2]Texas A&M University, [3]Kyoto Sangyo University)

Heat shock response is a major homeostatic mechanism for controlling the state of protein folding and degradation in all organisms. Expression of heat shock genes in *E. coli* is both under positive control by σ^{32}, a transcription factor dedicated to the heat shock response, and under negative feedback control (inactivation/degradation of σ^{32}) by stress-inducible molecular chaperones (DnaK/J-GrpE, GroEL/S). σ^{32} is extremely unstable *in vivo* and is degraded by membrane-localized protease FtsH. Chaperones contribute to rapid degradation of σ^{32} *in vivo*, whereas its degradation *in vitro* is very slow and not enhanced by chaperones. It is possible that some other factors are involved in the degradation of σ^{32} *in vivo*.

In collaboration with Drs. Takashi Yura and Carol A. Gross [1)], we identified the co-translational protein targeting machinery, comprised of the Signal Recognition Particle (SRP) and the SRP Receptor (SR; FtsY), as a regulator of σ^{32}. We showed that SRP directly binds to σ^{32},

that a population of σ^{32} is present in the membrane fraction and that both the SRP-dependent machinery and the σ^{32} region 2.1 involved in the feedback control are important for the localization of σ^{32}. The regulatory defects in heat shock response circuitry caused by mutations of either the σ^{32} region 2.1 or the co-translational targeting system are circumvented by artificially tethering σ^{32} to the membrane. We proposed that SRP-dependent membrane localization is a critical step in the control circuitry that governs the activity and stability of σ^{32}.

1) Lim, B., *et al*. (2013) Heat shock transcription factor σ^{32} co-opts the signal recognition particle to regulate protein homeostasis in *E. coli*. ***PLoS Biology*** **11**, e1001735

Molecular mechanisms of the enhancement of protein export by the membrane protein complex SecDF: K. MITO, A. MUKUNO, Y. MACHIDA, Y. AKIYAMA and H. MORI

The SecYEG translocon and the SecA ATPase cooperate to facilitate protein export across the bacterial cytoplasmic membrane. In addition to these essential core components, SecDF, a complex containing two membrane-integrated Sec factors, play important roles in efficient protein export *in vivo*. We determined the crystal structure of SecDF from *Thermus thermophilus* at 3.3 Å resolution and proposed a working hypothesis based on structure-instructed biochemical and biophysical studies[1)]. According to the model, SecDF forms a complex with SecYEG translocon, captures a substrate polypeptide emerging from the translocon by its P1 (the first periplasmic) domain and undergoes conformational changes using the PMF (proton motive force) to facilitate forward movement of the polypeptide. However, modes of interactions between SecDF and Sec-related factors including SecYEG and a substrate polypeptide remain largely unknown. To gain information on this issue, we performed systematic site-directed *in vivo* photo-cross-linking analysis[2)] targeted to *E. coli* SecD. Based on the *Thermus thermophilus* SecDF structure, we designed *E. coli* SecD mutations so as to introduce a photo reactive amino acid, *p*-benzoyl phenylalanine (pBPA) on the molecular surface of the protein. We constructed more than 80 SecD derivatives and carried out the cross-linking experiments with them. So far, we identified several *E.coli* SecD residues that are in close proximity to SecF, periplasmic chaperones or a substrate protein. In addition, possible intramolecular crosslinkings within the SecD P1 domain were detected. Interestingly, the amounts of some of the SecD P1-SecF intermolecular and the SecD P1 intramolecular crosslinked products were dramatically decreased, when cells expressing these SecD derivatives were treated with CCCP (a protonophore) prior to the UV-crosslinking to collapse PMF across the membrane. These results nicely fit our model that the proton conductance of SecDF somehow couples with the movement of the P1 domain.

1) Tsukazaki, T. *et al.* (2011) Structure and function of a membrane component SecDF that enhances protein export. ***Nature* 474,** 235-238.

2) Chin, J. W. *et al.* (2002) Addition of a photocrosslinking amino acid to the genetic code of *Escherichia coli.* ***Proc. Natl. Acad. Sci. USA.* 99**, 11020-11024.

List of Publications

Lim, B., Miyazaki, R.[a], Neher, S.[a], Siegele, D.A., Ito, K., Walter, P., Akiyama, Y.*, Yura, Y.*, and Gross, C.A.* (2013) Heat shock transcription factor σ^{32} co-opts the signal recognition particle to regulate protein homeostasis in *E. coli*. PLoS Biology *11*, e1001735.

[a] These authors contributed equally to this work. *Corresponding author

Narita, S.-i., Masui, C., Suzuki, T., Dohmae, N., and Akiyama, Y. (2013) Protease homolog BepA (YfgC) promotes assembly and degradation of β-barrel membrane proteins in *Escherichia coli*. Proc. Natl. Acad. Sci. USA *110,* E3612-E3621.

Ishii, E., Eguchi, Y., and Utsumi, R. (2013) Mechanism of activation of PhoQ/PhoP two-component signal transduction by SafA, an auxiliary protein of PhoQ histidine kinase in *Escherichia coli*. Biosci. Biotechnol. Biochem. *77,* 814-819.

Kroos, L., and Akiyama, Y. (2013) Biochemical and structural insights into intramembrane metalloprotease mechanisms. Biochim. Biopys. Acta *1828*, 2873-2885.

Ha, Y., Akiyama, Y., and Xue, Y. (2013) Structure and mechanism of rhomboid protease. J. Biol. Chem. *288*, 15430-15436.

Ogura, T., Okuno, T., Suno, R., and Akiyama, Y. (2013) FtsH Protease. Handbook of Proteolytic Enzymes 3rd ed. (ed. Rawlings, N. D. and Salvesen, G.) Elsevier Ltd. pp685-692.

Hizukuri, Y., Ito, K., and Akiyama, Y. (2013) RseP Peptidase. Handbook of Proteolytic Enzymes 3rd ed. (ed. Rawlings, N. D. and Salvesen, G.) Elsevier Ltd. pp1546-1550.

Akiyama, Y., and Ito, K. (2013) HtpX Peptidase. Handbook of Proteolytic Enzymes 3rd ed. (ed. Rawlings, N. D. and Salvesen, G.) Elsevier Ltd. pp683-685.

森 博幸、塚崎智也（2013）細菌のタンパク質分泌を促進する膜タンパク質 SecDF の構造

と機能　化学と生物 51、28-35.

江口陽子、加藤明宣、石井英治、内海龍太郎（2013）コネクターがつなぐ細菌情報伝達ネットワーク　化学と生物 51、241-249.

Mori, H., Tsukazaki. T., Machida, Y., Mito, K., Nureki, O., Ito, K., and Akiyama, Y.: Structure and function of SecDF, a membrane integrated protein translocation enhancing factor. 第 86 回日本細菌学会総会ワークショップ W7「細菌構造研究の進展開：分泌装置、細胞骨格、運動装置、細菌表層の構造体を中心に」、幕張、2013 年 3 月 19 日

成田新一郎、秋山芳展：大腸菌 βバレル型タンパク質の品質管理に関わるプロテアーゼホモログ BepA(YfgC) の機能解析、2012 年度国立遺伝学研究所研究会「単細胞システムの構築とその維持機構の研究」、三島、2013 年 3 月 28-29 日

森 博幸：ビブリオ菌 SecDF パラログの発現制御機構、2012 年度国立遺伝学研究所研究会「単細胞システムの構築とその維持機構の研究」、三島、2013 年 3 月 28-29 日

檜作洋平、禾 晃和、小田 隆、田畑早苗、川上-田村恵子、佐藤 衛、高木淳一、秋山芳展：大腸菌膜内切断プロテアーゼ RseP の基質認識における PDZ ドメインの役割、第 86 回日本生化学会大会、シンポジウム「非常識なプロテアーゼ反応：膜内部でのタンパク質切断」、横浜、2013 年 9 月 11-13 日

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秋山芳展：膜内部でのタンパク質切断による表層ストレス応答制御機構、京都大学微生物科学寄付研究部門主催第二回シンポジウム「微生物科学研究の多様性と新展開」、京都、2013 年 11 月 8 日

成田新一郎、鈴木健裕、堂前 直、秋山芳展：大腸菌 β バレル型膜タンパク質の生合成に関わる BepA (YfgC) の機能解析、日本農芸化学会 2013 年度（平成 25 年度）大会、仙台、2013 年 3 月 27 日

檜作洋平、小田 隆、田畑早苗、川上-田村恵子、佐藤 衛、高木淳一、禾 晃和、秋山芳展：膜内切断プロテアーゼ RseP のタンデム PDZ ドメインが介する切断基質選別機構、第 10 回

21 世紀大腸菌研究会、伊豆、2013 年 6 月 20-21 日

三登一八、町田裕紀子、塚崎智也、伊藤維昭、秋山芳展、森 博幸：部位特異的 in vivo 光架橋法によるタンパク質膜透過促進因子 SecDF の基質結合部位の探索、第 10 回 21 世紀大腸菌研究会、伊豆、2013 年 6 月 20-21 日

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舛井千草、成田新一郎、秋山芳展 ：部位特異的 in vivo 光架橋法による大腸菌ペリプラズムタンパク質 BepA(YfgC)の近接因子探索、第 10 回 21 世紀大腸菌研究会、伊豆、2013 年 6 月 20-21 日

Nogi, T., Hizukuri, Y., Oda, T., Tabata, S., Tamura-Kawakami, K., Sato, M., Takagi, J., and Akiyama, Y.: Structural analysis of the PDZ tandem fragment of the bacterial intramembrane-cleaving protease RseP. International Conference on Structural Genomics 2013, Sapporo, Japan, 29 July -21 August 2013.

宮﨑亮次、由良 隆、森 博幸、秋山芳展：大腸菌熱ショック転写因子 σ^{32} の膜への targeting を介した機能制御機構、第 86 回日本生化学会大会、横浜、2013 年 9 月 11-13 日

成田新一郎、舛井千草、鈴木健裕、堂前 直、秋山芳展：大腸菌プロテアーゼ BepA は外膜タンパク質の組立と分解を促進する、日本農芸化学会東北支部第 148 会大会、盛岡、2013 年 10 月 26 日

檜作洋平、小田 隆、田畑早苗、川上-田村恵子、佐藤 衛、高木淳一、禾 晃和、秋山芳展：Substrate discrimination mechanism by a PDZ tandem in the intramembrane protease RseP that regulates extracytoplasmic stress response. 第 51 回日本生物物理学会年会、京都、2013 年 10 月 28 日

宮﨑亮次：大腸菌熱ショック転写因子σ^{32} の膜への targeting を介した機能制御機構、京都大学微生物科学寄付研究部門主催第二回シンポジウム「微生物科学研究の多様性と新展開」、京都、2013 年 11 月 8 日

秋山光市郎：大腸菌の S2P ファミリー膜内切断プロテアーゼ RseP の「膜内部に挿入したβヘアピン領域」の機能、京都大学微生物科学寄付研究部門主催第二回シンポジウム「微生

物科学研究の多様性と新展開」、京都、2013 年 11 月 8 日

檜作洋平、小田 隆、田畑早苗、川上-田村恵子、佐藤 衛、高木淳一、禾 晃和、秋山芳展：立体構造解析に基づく大腸菌膜内切断プロテアーゼ RseP のタンデム PDZ ドメインによる切断基質選別機構モデル、第 36 回日本分子生物学会年会、神戸、2013 年 12 月 3 日

II. Second Group

Members

Associate Professor	**Hiroyuki Sakai**
Assistant Professor	**Shin-ichi Yanagawa**
Research Fellow	**Naoko Kajitani**

Topics

Analysis of Keratin-Associated Protein 13-Induced Activation of Canonical Wnt Signaling Pathway in vivo: S. YANAGAWA

I found that Keratin associated protein (Krtap) 13 binds to cytoplasmic portion of LRP6, a co-receptor for Wnt. Surprisingly, Krtap13 overexpression markedly stimulates Wnt signaling. Krtap13 was found to induce co-clustering of LRP6 and Dvls, thereby inhibiting Axin mediated b-catenin destruction complex that leads to activation of Wn signaling.
To analyze effect of ectopic overexpression of Krtap13 i*n vivo,* I generated a Krtap13-trans-gene (Krtap13-Tg) consisting of CAG-promoter, loxp-polyA-loxp cassette, and 3XFLAG-tagged human Krtap13 cDNA and transgenic mouse lines carrying this Tg were established. This Krtap13-Tg can express Krtap13 only after Cre-induced recombination of Tg. By crossing these Krtap13-Tg mice with another transgenic mice that express Cre in a tissue-specific way, I generated a mice system that allowed tissue specific overexpression of Krtap13. Twenty-five% of mice born from crossing between Krtap13-Tg mice and CAG-Cre-Tg mice developed Lymphoma/ Leukemia 1~1.5 year after birth. Lymphoma/ Leukemia cell typing analyses using FACS are underway.

Identification of Novel Function of Human Papillomavirus E4: N. KAJITANI and H. SAKAI

HPV infection begins in the basal cells of the epithelium, and as these cells divide, differentiate, and migrate toward the surface of the epithelium, the virus is able to complete its life cycle. The viral life cycle depends on the differentiation of the epithelium, but how the life cycle is controlled is not well understood. It is interesting that although viral oncoproteins cause the increase of cellular proliferation and/or transformation, terminally cellular differentiation of epithelium is required for completion of the viral life cycle.

The expression of E1^E4 occurs in the upper layers of the HPV-infected epithelium, coordinating with the onset of viral genome amplification and the expression of viral late genes. It is known that E1^E4 disrupts the keratin networks. It is also known that E1^E4 induces G_2/M cell cycle arrest. But it is yet to be known well about the details of E1^E4. To investigate novel functions of E1^E4, we performed yeast two-hybrid assays and got several candidate proteins as which interacts with E1^E4. As the results, it is suggested that E1^E4 associates with the Aggresome compartment that is one of cellular inclusion body systems. In the future, we will ascertain the function of E1^E4 and its involvement in the viral life cycle.

Analysis of CAF formation mechanism using HPV positive cells: H. SAKAI and N. KAJITANI

In many reports, the importance of the interaction between the cancer stem cells and the microenvironments has been indicated. In the previous studies, it was suggested that HPV E6, E7, c-Myc, and H-ras were the key factors for the establishment of the cancer stem cell in the cervical cancer. These factors might alter the microenvironment to be favorable for cancer development. To examine the effect of the cancer cells in fostering the cancer-associated fibroblasts (CAFs), HPV-positive cancer cells, SiHa, HeLa, and Caski, were applied to the organotypic raft culture, and the effects on the fibroblasts were analyzed by gene-expression profiling. The expressions of CD44 and α-SMA were used as the markers for the CAF induction. In another experiment, the fibroblasts expressing an oncogene, *myc*, *src*, or *ras* were used as the transformed fibroblasts, and normal HFKs or HeLa cells were overlaid on these cells. The effect of TGF・produced by CAFs on the EMT of normal and HPV-positive keratinocytes was also examined. These inter-cellular communications might be important for the progression of the cervical cancer.

List of Publications

Ma, G., Yasunaga, J.-I., Fan, J., Yanagawa, S.-I., Matsuoka, M. (2013). HTLV-1bZIP factor dysregulates the Wnt pathways to support proliferation and migration of adult T-cell leukemia cells. Oncogene, *32*, 4222-4230.

Kajitani N., Satsuka A., Yoshida S. and Sakai H. (2013). HPV18 E1^E4 is assembled into aggresome-like compartment and involved in sequestration of viral oncoproteins. Frontiers in Virology *4*: article 251.

柳川伸一（2013）20 年前の Wnt 研究の現場はどうだったか　細胞工学 32、406.

梶谷直子、酒井博幸：HPV E1^E4 はアグリソームを形成しウイルス因子のタンパク量制御に関与する、第 72 回日本癌学会学術総会、横浜、2013 年 10 月 3-5 日

梶谷直子、酒井博幸：Human papillomavirus (HPV) E1^E4 は aggresome を形成する、第 61 回日本ウイルス学会学術集会、神戸、2013 年 11 月 10-12 日

DEPARTMENT OF VIRAL ONCOLOGY
LABORATORY OF CELL REGULATION

Members

Professor	**Masahiko Sugita**
Assistant Professor	**Daisuke Morita**
Graduate Student	**Yuki Hattori**
	Yukie Yamamoto
	Satoru Murata
	Hiromi Tashiro
	Ayumi Miyamoto
	Takeharu Watanabe

Introduction

Presentation of protein-derived peptide antigens (Ags) by major histocompatibility complex (MHC)-encoded class I and class II molecules has been a central dogma in modern immunology. Peptide Ags bound to MHC molecules are recognized by T cells bearing αβ T-cell receptors (TCRs). However, the paradigm that Ag-specific T cell activation only involved recognition of peptide Ags turned out to be incorrect. Some T cells bearing αβ TCRs recognize lipid Ags in a group 1 CD1 (CD1a, -b, and -c)-dependent manner. Thus, the Ag-specific adaptive immune system comprises two separate pathways, one directed against peptide Ags (mediated by MHC molecules) and the other directed against lipid Ags (mediated by group 1 CD1 molecules). These two pathways function cooperatively to achieve highest levels of Ag-specific host defense.

Both MHC and CD1 pathways are equally important; however, only several laboratories, including ours, focus on the latter pathway. This is primarily due to the fact that mice and rats that are highly useful for immunological studies have deleted genes for group 1 CD1 family, and thus, lack the lipid recognition system that is comparable to that in humans. Therefore, we have developed three distinct but complementary animal models; namely, human CD1 transgenic (Tg) mice, guinea pigs, and rhesus monkeys. The human *CD1A* genome-Tg mice were established, in which immature thymocytes and epidermal Langerhans cells specifically expressed human CD1a proteins. Alternatively, we found guinea pigs invaluable for lipid immunity research as the animals have evolved the CD1 system that is comparable with that in humans. Finally, our laboratory has made efforts to utilize non-human primates, such as rhesus macaques. As described below, rhesus monkeys have now been analyzed extensively in our laboratory for lipid immunity to mycobacteria and retroviruses, resulting in identification of lipid-based vaccine candidates against tuberculosis

and discovery of viral lipopeptide-specific cytotoxic T lymphocyte (CTL) responses.

Topics

Lipid-specific immunity in tuberculosis: A. MIYAMOTO, D. MORITA, Y. HATTORI, T. Nakamura[1], T. igarashi[2], H. HARASHIMA[1] and M. SUGITA ([1]Hokkaido Univ., [2]Laboratory of Primate Model, IVR.)

Mycobacteria, such as *Mycobacterium tuberculosis*, possess highly lipid-rich cell walls that are critical not simply for their acid-fast properties but also their survival and replication. The cell wall contains mycolic acids (MAs), an α-alkyl-β-hydroxy fatty acid with extremely long carbon chains ($\sim C_{80}$), which are densely aligned in covalent association with the underlying arabinogalactan sugar layer. Arabinogalactan-linked MAs extend outward and interact non-covalently with carbon chains of the surface exposed mycolyl glycolipids, such as trehalose 6,6'-dimycolate (TDM), thereby forming the hydrophobic cell wall architecture that is unique to mycobacteria. Although this is a well accepted model, we reasoned that this could not represent pathogenic mycobacteria surviving within the host. TDM is an essential component of the Freund's adjuvant and a potent ligand for innate immunity receptors, such as the C-type lectin Mincle; therefore, pathogenic mycobacteria should have evolved an evasive maneuver to down-regulate TDM expression within the host in order to escape from the host innate immunity. Indeed, upon entry into the host, pathogenic mycobacteria produce glucose monomycolate (GMM), a glucosylated species of MAs, by utilizing host-derived glucose as a competitive substrate for mycolyltransferases that primarily catalyze the final step of TDM synthesis when glucose is absent **(*J. Biol. Chem.* 283: 28835-28841, 2008)**. Thus, GMM is a marker lipid for pathogenic mycobacteria sustaining active metabolism within the host. Strikingly, our acquired immune system is equipped with CD1-restricted T cells that recognize GMM and thus, is able to precisely monitor live infection with pathogenic mycobacteria.

We have obtained evidence for the delayed-type hypersensitivity (DTH) or type IV allergy to GMM both in guinea pigs and in rhesus monkeys that is associated with the up-regulated production of host-protective cytokines, such as IFN-γ and TNF-α **(*J. Biol. Chem.* 286: 16800-16806, 2011; *Infect. Immun.* 81: 311-316, 2013)**. More importantly, our recent studies have indicated that an intracutaneous vaccination of guinea pigs with GMM alone is able to confer host resistance to mycobacterial infections (unpublished), raising the possibility that GMM could be utilized as a new type of lipid-based vaccines against human tuberculosis. We have completed the formulation of GMM/adjuvant vaccines that are capable of inducing GMM-specific T cell

responses efficiently in rhesus monkeys and thus, are potentially applicable to humans (unpublished).

Lipopeptide-specific immunity in AIDS: D. MORITA, Y. YAMAMOTO, H. TASHIRO, J. SUZUKI[1], N. MORI[2], T. IGARASHI[3], and M. SUGITA ([1]Primate Research Inst., Kyoto Univ., [2]Graduate Sch. Agriculture, Kyoto Univ., [3]Laboratory of Primate Model, IVR,)

By taking full advantage of IVR's superb monkey research environments and by fostering intra-institutional collaborations with Prof. Igarashi's laboratory and inter-institutional collaboration with the Primate Research Institute, we are encouraged to address a naive question as to how lipid immunity functions in host defense against viral infections as viruses do not express their own lipids. Given that some of the viral proteins require modification with host-derived fatty acids for their critical function, we hypothesized that the host immunity might be able to detect lipidated viral proteins (lipoproteins). Indeed, we found that rhesus macaque monkeys infected with the simian immunodeficiency virus (SIV) mounted cytotoxic T lymphocyte responses to N-myristoylated SIV Nef 5-mer lipopeptide (C14nef5) **(*J. Immunol.* 187: 608-612, 2011)**. Functional studies with C14nef5-derived structural analogues revealed that the putative lipopetide Ag-presenting molecule might have two separate Ag-binding sites, one for interaction with a C_{14} saturated acyl chain and the other for anchorage of the C-terminal serine residues **(*J. Virol.* 87: 482-488, 2013)**. We are now successful in determining the molecular identity of the lipopeptide Ag-presenting molecule (unpublished).

List of Publications

Morita, D., Yamamoto, Y., Suzuki, J., Mori, N., Igarashi, T., Sugita, M. (2013). Molecular Requirements for T Cell Recognition of N-Myristoylated Peptides Derived from the Simian Immunodeficiency Virus Nef Protein. J Virol. *87*, 482-488.

Morita, D., Hattori, Y., Nakamura, T., Igarashi, T., Harashima, H., Sugita, M. (2013). Major T cell response to a mycolyl glycolipid is mediated by CD1c molecules in rhesus macaques. Infect Immun. *81*, 311-316.

Morita, D., Miyamoto, A., Hattori, Y., Komori, T., Nakamura, T., Igarashi, T., Harashima, H., Sugita, M. (2013). Th1-skewed tissue responses to a mycolyl glycolipid in mycobacteria-infected rhesus macaques. Biochem Biophys Res Commun. *441*, 108-113.

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杉田昌彦：CD1 と獲得免疫、第 63 回日本アレルギー学会秋季学術大会、東京、2013 年 11 月 28-30 日

森田大輔、杉田昌彦：Lipopeptide Ag presentation by MHC class I molecules: evidence from SIV-infected monkeys. 第 42 回日本免疫学会学術集会、千葉、2013 年 12 月 11-13 日

DEPARTMENT OF VIRAL ONCOLOGY
LABORATORY OF TUMOR BIOGENESIS

Members

Professor	**Shin Yonehara**
Assistant Professor	**Akira Murakami**

Introduction

Apoptosis, or programmed cell death, plays an important role in many biological processes, including embryogenesis, development of immune system, maintenance of tissue homeostasis, and elimination of virus-infected and tumor cells. We found cell surface Fas antigen (Fas), which can directly mediate apoptosis-inducing signals into cells by stimulation with agonistic anti-Fas mAbs or Fas ligand. Our main research project is to understand the intracellular signal transduction mechanism of cell death including apoptosis and caspase-independent novel types of cell death, and the biological significance/physiological role of cell death and cell death-regulating molecules. Investigations of molecular mechanisms and physiological roles of cell death are important for a better understanding of mammalian immune system, embryogenesis and tumorigenesis.

Topics

Identification of a novel type 2 innate immunocyte with ability to enhance IgE production: A. FUKUOKA, S. FUTATSUGI-YUMIKURA, S. TAKAHASHI, H. KAZAMA, T. IYODA, T. YOSHIMOTO, K. INABA, K. NAKAHISHI and S. YONEHARA

Fas (CD95), a member of the TNF receptor super family, mediates apoptosis-inducing signals in its expressing cells, especially in self-reactive cells. We recently reported that $Fas^{-/-}$ mice with a BALB/c background (BALB/c $Fas^{-/-}$ mice) developed blepharitis with allergic inflammation that was accompanied by hyper IgE production. Here, we found a novel type of immunocyte in the spleen of BALB/c $Fas^{-/-}$ mice, which enhanced the production of IgE by B cells in the presence of IL-4 and CD40 signaling *in vitro*. The immunocyte did not express lineage markers, but expressed Thy-1 and Sca-1 just like recently identified type 2 innate lymphoid cells, such as natural helper (NH) cells and nuocytes. However, they did not express c-Kit, IL-7R and IL-33R (T1/ST2), important markers of type 2 innate lymphoid cells. Instead, our identified $Lin^{-}Thy\text{-}1^{+}Sca\text{-}1^{+}$ cells

expressed IL-18R, and secreted Th2 cytokines when co-cultured with B cells or stimulated with IL-18 and IL-2. Moreover, we found essentially the same type of cells in BALB/c wild-type mice as in BALB/c $Fas^{-/-}$ mice, which expressed Fas and enhanced IgE production in contact with B cells *in vitro*. Collectively, the newly identified $Lin^{-}Thy\text{-}1^{+}Sca\text{-}1^{+}$ cell, which we designated a F-NH cell (Fas-expressing natural helper cell), is a novel type 2 innate immunocyte with activity to enhance IgE production from B cells with the help of IL-4 and CD40 signaling. F-NH cells may play an important role in the development of chronic allergic inflammation.

FLASH interacts with the LSD1/CoREST1/HDAC1 complex: W.F. KUANG, M. KIRIYAMA, M. SAITO, K.K. LEE, F. ISHIKAWA and S. YONEHARA

FLICE-associated huge protein (FLASH) /CASP8AP2 is a multifunctional protein that has been linked to transcriptional control, S phase progression, and histone pre-mRNA processing. To further understand the functions of FLASH, we used mass spectrometry to identify the proteins co-immunoprecipitated with Flag-tagged FLASH and identified histone lysine-specific demethylase 1 (LSD1) and corepressor of REST 1 (CoREST1), both of which are members of the LSD1/CoREST1/HDAC corepressor complex. The interaction domain of FLASH with LSD1 or CoREST1 was mapped to the central region of FLASH. Although LSD1 and CoREST1 interacted with each other, both could directly and independently bind to FLASH. The downregulation of either LSD1 or CoREST1 expression did not interfere with the interaction of each molecule with FLASH. Both LSD1 and CoREST1 formed nuclear foci that co-localized with FLASH *in vivo*. The overexpression of FLASH resulted in an increase in dimethyl histone H3 K4 **(**H3K4) levels, whereas the methylation levels of trimethyl H3K4, dimethyl H3K9, and trimethyl H3K9 remained unchanged. These results indicate that FLASH may modulate histone methylation by interacting with the LSD1/CoREST1 complex.

IFN-γ triggers RIP1/RIP3-mediated programmed necrosis in human and mouse monocyte-derived cell lines: Y. Mori and S. YONEHARA

Stimulation of death receptor family members such as TNF receptor or Fas (CD95/Apo-1) generally induces apoptosis; however, necrotic cell death, called necroptosis, can be induced when the activity of caspase-8 is impaired. Recently, programmed necrosis was reported to be induced by treatment with not only TNF-α or FasL but also LPS, poly(I:C) or etoposide through activation of receptor-interacting protein kinase 1 (RIP1) and RIP3. We also found that interferon-γ (IFN-γ) induces RIP3-dependent programmed necrosis in caspase-8 KO MEFs. However, it remains unclear whether IFN-γ can induce programmed necrosis in other types of cells. Here, we show that human monocytic leukemia-derived U937 cells and mouse macrophage-derived RAW264.7 cells are

susceptible to IFN-γ-induced cell death in the presence of zVAD-fmk, a pan-caspase inhibitor. The IFN-γ-induced necrotic cell death was not accompanied with the feature of apoptosis; cleavage of caspase-3 and PARP, and pre-exposure of phosphatidylserine to the cell surface. In the same manner as TNF-α-induced necrosis, IFN-γ-induced cell death was inhibited by pretreatment with necrostatin-1, an inhibitor of RIP1 kinase. On the other hand, inhibition of protein synthesis by co-treatment with cycloheximide notably inhibited IFN-γ-induced cell death, but significantly enhanced TNF-α-induced apoptosis and necrosis. RAW264.7 cells expressing a specific shRNA to RIP3 or MLKL were fully protected from IFN-γ-induced cell death. In contrast to wild-type RIP3, kinase mutant or RHIM mutant RIP3 failed to induce necrosis. All the results indicate that the IFN-γ-induced cell death is a novel type of programmed cell death mediated by new gene expression and RIP1/RIP3-MLKL signaling.

A role of Wnt signals in the differentiation of mouse ES cells: A. MURAKAMI

ES cells are maintained in an undifferentiated state or are induced to differentiated cells under various culture conditions. Many signaling pathways or factors have been identified to be involved in those processes. Among them, we are currently interested in the Wnt signaling pathway, which is likely to contribute to both processes. An activation of the Wnt signal keeps ES cells in undifferentiated state. On the other hand, there are some Wnt signals that are involved in an induction of differentiation.

In ES cells, an expression of several members of the Wnt family was detected, such as Wnt1, Wnt3, Wnt3a, Wnt6, Wnt8a and Wnt10b. Among them, Wnt3 and Wnt8a seem to be involved in mesoderm induction through an activation of Brachyury expression, one of key factors for the mesoderm induction. Their expression patterns during the differentiation were similar to that of Brachyury, and knock down of either gene expression specifically inhibited the mesoderm induction. Analysis of a precise mechanism by which the Wnt signals contribute to the mesoderm induction process is underway.

List of Publications

Fukuoka, A., Futatsugi-Yumikura, S., Takahashi, S., Kazama, H., Iyoda, T., Yoshimoto, T., Inaba, K., Nakanishi, K., and Yonehara, S. (2013). Identification of a novel type 2 innate immunocyte with ability to enhance IgE production. Int Immunol *25*, 373-382.

Takahashi, S., Futatsugi-Yumikura, S., Fukuoka, A., Yoshimoto, T., Nakanishi, K., and Yonehara, S. (2013). Fas deficiency in mice with the Balb/c background induces blepharitis with allergic

inflammation and hyper-IgE production in conjunction with severe autoimmune disease. Int Immunol *25*, 287-293.

Nakanishi, Y., Seno, H., Fukuoka, A., Ueo, T., Yamaga, Y., Maruno, T., Nakanishi, N., Kanda, K., Komekado, H., Kawada, M., Isomura, A., Kawada, K., Sakai, Y., Yanagita, M., Kageyama, R., Kawaguchi, Y., Taketo, M.M., Yonehara, S., and Chiba, T. (2013). Dclk1 distinguishes between tumor and normal stem cells in the intestine. Nat Genet *45*, 98-103.

Uchiyama, R., Yonehara, S., and Tsutsui, H. (2013). Fas-mediated inflammatory response in *Listeria monocytogenes* infection. J Immunol *190*, 4245-4254.

米原 伸（2013）【企画】細胞死 Update：基礎から臨床までを俯瞰して　医学のあゆみ 第1土曜特集 246.

米原 伸 (2013) はじめに、細胞死 Update : 基礎から臨床までを俯瞰して　医学のあゆみ 第1土曜特集 246、353.

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米原 伸：がん細胞特異的機能分子FLASH／Casp8ap2の機能および レチノイン酸シグナルを調節する新しい分子機構について、平成25年度 第2回芝蘭会産学情報交流会、京都市、2013年11月8日

Shota Sakaguchi, Shunsuke Kuroki, and Shin Yonehara: Analysis of the molecular mechanism of a novel type of programmed necrosis induce by interferon-γ. The 11th International Student Seminar, Kyoto, 5-6 March 2013.

森 勇貴、米原 伸：IFN-γはヒトおよびマウス単球由来細胞株にプログラムされたネクローシスを引き起こす、第36回日本分子生物学会年会、神戸市、2013年 12月3-6日

DEPARTMENT OF VIRAL ONCOLOGY
LABORATORY OF HUMAN TUMOR VIRUSES

I. First Group

Members

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	Shoko Nakamura
	Shohei Kojima
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Introduction

The researches carried out in this group are focused on RNA viruses, especially negative strand RNA viruses replicating in the cell nucleus, such as bornavirus and influenza virus. All our projects aim to understand the fundamental mechanisms of the replication and pathogenesis of the viruses. In current researches we are investigating the replication and persistent mechanism of the bornavirus in the cell nucleus. The understanding the biological significance of the endogenous element of bornavirus nucleoprotein (EBLN) in mammalian genomes is one of the main focuses of bornavirus researches.

Topics

1) Comprehensive analysis of endogenous bornavirus-like elements in eukaryote genomes: K. TOMONAGA.

Bornaviruses are the only animal RNA viruses that establish a persistent infection in their host cell nucleus. Studies of bornaviruses have provided unique information about viral replication strategies and virus–host interactions. Although bornaviruses do not integrate into the host genome during their replication cycle, we and others have recently reported that there are DNA sequences

derived from the mRNAs of ancient bornaviruses in the genomes of vertebrates, including humans, and these have been designated endogenous borna-like (EBL) elements. Therefore, bornaviruses have been interacting with their hosts as driving forces in the evolution of host genomes in a previously unexpected way. Studies of EBL elements have provided new models for virology, evolutionary biology and general cell biology. In this review, we summarize the data on EBL elements including what we have newly identified in eukaryotes genomes, and discuss the biological significance of EBL elements, with a focus on EBL nucleoprotein elements in mammalian genomes. Surprisingly, EBL elements were detected in the genomes of invertebrates, suggesting that the host range of bornaviruses may be much wider than previously thought. We also review our new data on non-retroviral integration of Borna disease virus.

2) Inhibition of BDV replication by an endogenous bornavirus element in ground squirrel genome: K. FUJINO, M. HORIE, T. HONDA and K. TOMONAGA.

Animal genomes contain endogenous viral sequences, such as endogenous retroviruses. Recently, we and others discovered that non-retroviral viruses (NRV) have also been endogenized in many vertebrate genomes. Bornavirus belongs to the Mononegavirales and have left endogenous elements, called EBLN, in the genomes of many mammals. The striking features of EBLN are that they hold relatively long open reading frame and show high sequence homology to the nucleoprotein (N) of current bornaviruses. Furthermore, it has been known that some EBLNs are transcribed as mRNA. These features of EBLNs provide us to speculate that EBLNs might have functions in the cells as adopted genes. The EBLN in the thirteen-lined ground squirrel (TLS) genome is the one of the most intriguing EBLNs, because the TLS EBLN exhibits 77% sequence identity to bornavirus N. To analyze the possible function of TLS EBLN, we revived TLS EBLN from the ground squirrel genomes and investigated the roles of the revived protein in Borna disease virus (BDV) replication. Interestingly, TLS EBLN co-localized with the viral factory of BDV in nucleus. In addition, TLS EBLN appeared to affect BDV polymerase activity by being incorporated into the viral ribonucleoprotein. Moreover, cell lines stably expressing TLS EBLN showed the resistance to BDV infection. Our results suggested that TLS EBLN may also have potential to inhibit the infection of related exogenous viruses.

3) Interaction between viral RNP and host factors in the nucleus: T. HONDA, S. KOJIMA, S. NAKAMURA, A. MAKINO and K. TOMONAGA.

BDV, a nonsegmented, negative-strand RNA virus, is characterized highly neurotropic and noncytopathic infection. BDV has several unique features. The most striking feature of BDV is that it establishes a long-lasting persistent infection in the cell nucleus without overt cytopathic effects.

This characteristic makes BDV the only animal RNA virus capable of intranuclear parasitism. Therefore, the study of BDV allows us to uncover previously unknown interactions between RNA virus and host factors. Recently, we demonstrated that BDV ribonucleoprotein (RNP) interacts directly with a host chromatin-binding protein, high mobility group box protein 1 (HMGB1), which influences BDV replication and persistent infection. To further investigate the role of HMGB1 in BDV persistence, we isolated HMGB1-binding proteins (HBPs) from the nucleus of BDV-infected cells. We identified that HBP-1, one of HBPs, associated with HMGB1 and BDV RNP in the nucleus. Knockdown of HBP-1 enhanced BDV replication, suggesting that HBP-1 represses BDV replication. Furthermore, we demonstrated that knockdown of HBP-1 decreased the formation of BDV RNP speckles in the cytosol. These results suggest that HBP-1 might translocate BDV RNP into the cytosol, resulting in the repression of BDV replication. Our data may provide a novel mechanism for regulation of RNA virus replication in the nucleus.

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牧野晶子、藤野 寛、平井悠哉、惣福 梢、中村祥子、小嶋将平、本田知之、朝長啓造：ボルナ病ウイルス N タンパク質のアンキリン様配列を介した NF-κB 活性化阻害、第 61 回日本ウイルス学会学術集会、神戸、2013 年 11 月 10-12 日

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藤野 寛、堀江真行、本田知之、朝長啓造：ジリスゲノムより復元した内在性ボルナウイルスはボルナ病ウイルスの感染を阻害する、第 61 回日本ウイルス学会学術集会、神戸、2013 年 11 月 10-12 日

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中村祥子、堀江真行、安木真世、大道寺智、久野 敦、奥崎大介、牧野晶子、本田知之、成松 久、中屋隆明、朝長啓造：A 型インフルエンザウイルス感染におけるムチン型糖転移酵素 GALNT3 の機能解析、第 61 回日本ウイルス学会学術集会、神戸、2013 年 11 月 10-12 日

中村祥子、堀江真行、安木真世、大道寺智、久野 敦、奥崎大介、牧野晶子、本田知之、成松 久、中屋隆明、朝長啓造：A型インフルエンザウイルス感染におけるムチン型糖転移酵素GALNT3の機能解析、第36回日本分子生物学会年次会、神戸、2013年12月3-6日

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II. Second Group

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	Yuichi Akahori
	Hitomi Okamura
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Assistant	**Eri Toyama**

Introduction

The researches carried out in this group are focused on hepatitis viruses, hepatitis B virus and hepatitis C virus, which cause chronic liver diseases, such as chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Our projects aim to reveal the lifecycles of these viruses, the interaction between human hepatocytes and these viruses at the molecular level. Development of novel anti-viral strategies based on our research outcomes is also intended. Understanding the pathogenesis of liver cancer and the liver differentiation using hepatic stem cells are also with a scope of our research purposes.

Topics

1) Thromboxane A_2 synthase inhibitors prevent production of infectious hepatitis C virus in thromboxane A_2 receptor independent manner: Y. Abe, H. Hasegawa, M. IMAMURA, N. HIRAGA, T. WAKITA, K. SHIMOTOHNO, K. CHAYAMA, M. HIJIKATA

Previously, we developed the three-dimensional (3D) cell culture system, using human immortalized hepatocytes (HuS-E/2 cells), supporting the lifecycle of blood-borne hepatitis C virus (bbHCV). In this culture system, infectious HCV were produced only from 3D-cultured HuS-E/2 cells. Thus, comparing gene expression profiles between 2D- and 3D-cultured HuS-E/2 cells, we have identified a novel host factor, thromboxane A_2 synthase (TXAS) that plays an important role in infectious HCV particle production.

To study the functional role of TXAS, we examined the effects of agonist and antagonist of TXA_2 receptor (TP). However, TP agonist and antagonist did not affect infectious HCV production

at all. And the TP mRNA expression was not observed in the cell lines used for recombinant HCV (HCVcc) production, and liver tissue from HCV-infected mice. In addition, we found that the TP-dependent signaling is deficient in cell lines used for HCVcc production. All these data suggested that TXAS inhibitor prevent production of infectious hepatitis C virus in TXA_2 receptor independent manner.

We previously observed that TXAS inhibitor blocked HCV expansion in drug treated chimeric mice with human liver at early stage of post-infection, but its effect waned over time, suggesting the probable appearance of drug resistant mutant HCV. Therefore, we performed the secondary infection experiments using sera from those mice, and found that the no suppressive effect of TXAS inhibitor on the expansion of secondary inoculated HCV, indicating the appearance of TXAS inhibitor resistant strains. So, we are now studying the characteristic feature of TXAS inhibitor resistant strains to reveal the molecular mechanism of infectious HCV production related with TXAS.

2) Type I and type III Interferons play anti-viral roles cooperatively in human hepatocytes to prevent early infection spread of viruses: Y. Tsugawa, H. Kato, T. FUJITA, K. SHIMOTOHNO, M. HIJIKATA

Hepatitis C virus (HCV) is known to infect human liver and cause the hepatitis, but the interferon (IFN) response, a first-line defense against viral infection, of virus-infected hepatocytes has not been defined clearly yet. Previously, we have reported that IFN-αιconstitutively produced in human in human hepatocytes plays a role of priming antiviral response. We also observed rapid induction of type III IFN in the cells immediately after RNA viral infection. However, we did not know how type III IFN interacts with type I IFN in terms of antiviral innate immune system. In this study, we investigated the interaction between type I and type III IFN responses in human hepatocytes at early phase of viral infection. Using immortalized human hepatocytes (HuS-E/2 cells), which preserve intact innate immunity, we examined the roles of those signals by silencing the signal from each IFN receptor. As the results, we found that only when both IFN receptor signalings were repressed, the clear reduction of the gene expression induced by virus infection, including IFN-α, IFN-β, IFN-λ3, IRF-7, and RIG-I genes was caused. The anti-RNA viral effects of those IFN were also assessed similarly. Simultaneous inhibition of those signals resulted in enhanced viral replication efficiently at 9 hrs post-infection, although the single inhibition showed lesser effect. These results suggest that type I and type III IFN signals in combination operate in human hepatocytes to prevent early infection spread of viruses including HCV. Further studies will help to understand the functional roles of these signals in anti-viral responses of human hepatocytes.

3) Construction of Hepatitis C Virus Infected Cell-Labeling System: Y. AKAHORI, Y.

KUSHIMA, H. OKAMURA, M. HIJIKATA

Hepatitis C Virus (HCV) is the major causative agent of hepatocellular carcinoma in Japan. HCV cell-culture systems have been developed by using human hepatoma-derived HuH-7 cells and recombinant HCV genomes and utilized in the study of HCV lifecycle. However, the mechanism of its persistent infection and the role of HCV in the hepatocellular carcinogenesis remain unclear. In order to study these issues, we aimed to build a system to label and isolate the cells persistently infected with HCV.

This system was built up with the reporter gene to label the infected cells and "sensor" protein to detect the HCV NS3/4A protease activity. The "sensor" comprising a transcriptional activator (TA) was designed to localize on the intracellular membrane through its trans-membrane domain (TMD). After HCV infection, TA domain is cleaved off from the "sensor" by NS3/4A protease. The released TA enters into nucleus and induces the reporter gene expression via the TA specific transcriptional promoter. Thus far, the "sensor" was constructed by using the HTLV-1 TAX or GAL4-VP16, the peptide sequence of IPS-1, and a part of the HCV NS2, as domains of TA, the target of NS3/4A protease, and TMD, respectively. As the reporter gene, the *EGFP* or *Gaussia luciferase* (*GLuc*) gene was used. When the Huh-7.5 cells transduced with the system were infected with recombinant HCV, GLuc activity in the culture supernatant was increased significantly. Furthermore, this activity was reduced by the addition of anti-CD81 antibody that inhibits HCV infection.

List of Publications

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Abe Y., Aly H.H., Hiraga N., Imamura M., Wakita T., Shimotohno K., Chayama K., Hijikata M.: Thromboxane A2 Synthase Inhibitors Prevent Production of Infectious Hepatitis C Virus. 20th International symposium on hepatitis C virus and related viruses. Melbourne, Australia, Oct 6-10, 2013.

Tsugawa Y, Kato, H. Fujita T., Shimotohno K., Hijikata M.: Type I and type III interferon play anti-viral roles cooperatively in human hepatocytes to prevent early infection spread of viruses. 20th International symposium on hepatitis C virus and related viruses. Melbourne, Australia, Oct 6-10, 2013.

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阿部雄一、アリ・ハッサン・フセイン、平賀伸彦、今村道雄、脇田隆字、下遠野邦忠、茶山一彰、土方 誠：トロンボキサン A_2 合成酵素は感染性 HCV 粒子形成を制御する、第 9 回広島肝臓プロジェクト研究センターシンポジウム、広島、2013 年 6 月 29 日

津川陽司、加藤博己、藤田尚志、下遠野邦忠、土方 誠：ヒト肝細胞の抗ウイルス初期応答においてⅠ型およびⅢ型インターフェロンは協調的に作用する、第 61 回日本ウイルス学会学術集会、神戸、2013 年 11 月 10-12 日

阿部雄一、長谷川輝、アリ・ハッサン・フセイン、平賀伸彦、今村道雄、脇田隆字、下遠野邦忠、茶山一彰、土方 誠：トロンボキサン A_2 合成酵素は C 型肝炎ウイルスの感染性粒子形成を制御する、第 36 回日本分子生物学会年会、神戸、2013 年 12 月 3-6 日

赤堀祐一、岡村 瞳、久島透嘉、土方 誠：C型肝炎ウイルス感染検出培養細胞系の開発、第36回日本分子生物学会年会、神戸、2013年12月3-6日

DEPARTMENT OF GENETICS AND MOLECULAR BIOLOGY
LABORATORY OF MOLECULAR GENETICS

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Introduction

We have been studying on antiviral innate immunity, particularly on the mechanism of type I interferon (IFN) gene regulation. 10 years ago, we identified viral RNA sensors, collectively termed as RIG-I-Like receptor. We have been focusing on the mechanism how RLR recognizes non-self RNA from self RNA. We also try to understand how viral replication within the cells is

sensed and triggers signals to activate antiviral program, by live cell imaging. We study different viruses including Polio, Influenza A, SFTS, hepatitis B viruses in the context of antiviral immune responses of the host.

Topics

Functional Characterization of Domains of IPS-1 Using an Inducible Oligomerization System: TAKAMATSU, S., ONOGUCHI, K., ONOMOTO, K., NARITA, R., TAKAHASI, K., ISHIDATE, F., FUJIWARA, T.K., YONEYAMA, M., KATO, H. AND FUJITA, T.

The innate immune system recognizes viral nucleic acids and stimulates cellular antiviral responses. Intracellular detection of viral RNA is mediated by the Retinoic acid inducible gene (RIG)-I Like Receptor (RLR), leading to production of type I interferon (IFN) and pro-inflammatory cytokines. Once cells are infected with a virus, RIG-I and MDA5 bind to viral RNA and undergo conformational change to transmit a signal through direct interaction with downstream CARD-containing adaptor protein, IFN-β promoter stimulator-1 (IPS-1, also referred as MAVS/VISA/Cardif). IPS-1 is composed of N-terminal Caspase Activation and Recruitment Domain (CARD), proline-rich domain, intermediate domain, and C-terminal transmembrane (TM) domain. The TM domain of IPS-1 anchors it to the mitochondrial outer membrane. It has been hypothesized that activated RLR triggers the accumulation of IPS-1, which forms oligomer as a scaffold for downstream signal proteins. However, the exact mechanisms of IPS-1-mediated signaling remain controversial. In this study, to reveal the details of IPS-1 signaling, we used an artificial oligomerization system to induce oligomerization of IPS-1 in cells. Artificial oligomerization of IPS-1 activated antiviral signaling without a viral infection. Using this system, we investigated the domain-requirement of IPS-1 for its signaling. We discovered that artificial oligomerization of IPS-1 could overcome the requirement of CARD and the TM domain. Moreover, from deletion- and point-mutant analyses, the C-terminal Tumor necrosis factor Receptor-Associated Factor (TRAF) binding motif of IPS-1 (aa. 453–460) present in the intermediate domain is critical for downstream signal transduction. Our results suggest that IPS-1 oligomerization is essential for the formation of a multiprotein signaling complex and enables downstream activation of transcription factors, Interferon Regulatory Factor 3 (IRF3) and Nuclear Factor-κB (NF-κB), leading to type I IFN and pro-inflammatory cytokine production.

Virus-induced expression of retinoic acid inducible gene-I and melanoma differentiation-associated gene 5 in the cochlear sensory epithelium: HAYASHI, Y.,

ONOMOTO, K., NARITA, R., YONEYAMA, M., KATO, H., NAKAGAWA, T., ITO, J., TAURA, A. AND FUJITA, T.

The inner ear has been regarded as an immunoprivileged site because of isolation by the blood-labyrinthine barrier. Several reports have indicated the existence of immune cells in the inner ear, but there are no reports showing immunocompetence of the cochlear tissue. In this report, we examined the potential involvement of retinoic acid inducible gene-I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5), which are critical for initiating antiviral innate immune responses. We found that RIG-I and MDA5 are expressed in the mouse cochlear sensory epithelium, including Hensen's and Claudius' cells. Ex vivo viral infection using Theiler's murine encephalomyelitis virus revealed that the virus replicates in these cells and that protein levels of RIG-I and MDA5 are up-regulated. Furthermore, the critical antiviral transcription factor, interferon (IFN) regulatory factor-3, is activated in the infected cells as judged by its nuclear translocation and the accumulation of type I IFN transcripts. These results strongly suggest that RIG-I and MDA5 participate in innate antiviral responses in cochlear tissue.

Encephalomyocarditis Virus Disrupts Stress Granules, the Critical Platform for Triggering Antiviral Innate Immune Responses: NG, C-S., JOGI, M., YOO, J-S., ONOMOTO, K., KOIKE, S., IWASAKI, T., YONEYAMA, M., KATO, H. AND FUJITA, T.

In response to stress, cells induce ribonucleoprotein aggregates, termed stress granules (SGs). SGs are transient loci containing translation-stalled mRNA, which is eventually degraded or recycled for translation. Infection of some viruses including influenza A virus with a deletion of non-structural protein 1 (IAVΔNS1) induces SG-like protein aggregates. Previously, we showed that IAVΔNS1-induced SGs are required for efficient induction of type I interferon (IFN). Here, we investigated SG formation by different viruses using GFP-tagged Ras-GAP SH3 domain binding protein-1 (GFP-G3BP1) as an SG probe. HeLa cells stably expressing GFP-G3BP1 were infected with different viruses and GFP fluorescence was monitored live with time-lapse microscopy. SG formation by different viruses was classified into 4 different patterns: no SG formation, stable SG formation, transient SG formation and alternate SG formation. We focused on EMCV infection, which exhibited transient SG formation. We found that EMCV disrupts SGs by cleavage of G3BP1 at late stages of infection (>8 h) through a similar mechanism to that by poliovirus. Expression of a G3BP1 mutant, which is resistant to the cleavage, conferred persistent formation of SG as well as an enhanced induction of IFN and other cytokines at late stages of infection. Additionally, knockdown of endogenous G3BP1 blocked SG formation with attenuated induction of IFN and potentiated viral replication. Taken together, our findings suggest a critical role of SG as an antiviral

platform and shed light on one of the mechanisms by which a virus interferes with host stress and subsequent antiviral responses.

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DEPARTMENT OF GENETICS AND MOLECULAR BIOLOGY
LABORATORY OF BIOCHEMISTRY

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Professor	**Mutsuhito Ohno**
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	Hiroto Izumi
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Introduction

In eukaryotic cells, many genes are separated by introns into multiple exons that should be joined together. In addition, the cell itself is separated by the nuclear envelope into two major compartments, the nucleus and the cytoplasm. These two types of separations necessitate specific gene expression mechanisms such as RNA splicing and nuclear transport. Prof. Mutsuhito OHNO's laboratory is studying various aspects of eukaryotic gene expression with great emphasis on "RNA" as a key molecule. In addition, Kitabatake's subgroup is focusing on quality control mechanisms of eukaryotic ribosome particles.

Topics

1) RNA distribution in the cell:

1-1) p54nrb/ NonO and PSF promote U snRNA nuclear export by accelerating its export complex assembly

The assembly of spliceosomal U snRNPs in metazoans requires nuclear export of U snRNA precursors. Four factors, nuclear cap-binding complex (CBC), phosphorylated adaptor for RNA export (PHAX), the export receptor CRM1, and RanGTP, gather at the m^7G-cap-proximal region and form the U snRNA export complex. Here we show that the multi-functional RNA-binding proteins p54nrb/NonO and PSF are U snRNA export stimulatory factors. These proteins, likely as a heterodimer, accelerate the recruitment of PHAX, and subsequently CRM1 and

Ran onto the RNA substrates *in vitro*, which mediates efficient U snRNA export *in vivo*. Our results reveal a new layer of regulation for U snRNA export and, hence, spliceosomal U snRNP biogenesis.

1-2) Conflicts between HIV-specific factors and the host factors in the viral RNA export

Intron-containing mRNA precursors are normally not exported to the cytoplasm but are retained in the nucleus until they are spliced to generate mature mRNAs. However, the AIDS virus, HIV-1 facilitates export of unspliced or partially-spliced viral RNAs by the action of the Rev protein that are encoded in the viral genome. This is achieved by switching the export pathways from normal cellular mRNA export (cellular type) pathway to the NES-dependent (virus type) pathway. In this research project, we attempt to understand the molecular mechanisms how the conflicts between the two export pathways are solved. To know that, we constructed a model RNA that mimicked partially-spliced viral RNA and microinjected it to the nucleus of *Xenopus* oocytes to examine its export pathway. We confirmed Rev changed the export pathway of the model RNA from cellular type to virus type. Moreover, we found Rev defines the export pathway not only by committing the virus type but also inhibiting the cellular type. That is to say, it is possible that Rev associates the cellular export factors on an identical RNA and the association may solve the conflicts between the two export pathway. We are focusing on physical and functional association between Rev and cellular export factors to understand the molecular mechanisms in detail.

1-3) hDbr1 is a nucleocytoplasmic shuttling protein with a protein phosphatase-like motif essential for debranching

In higher eukaryotes most genes contain multiple introns. Introns are excised from pre-mRNAs by splicing and eventually degraded in the nucleus. It is likely that rapid intron turnover in the nucleus is important in higher eukaryotes, but this pathway is poorly understood. In order to gain insights into this pathway, we analyzed the human lariat RNA debranching enzyme1 (hDbr1) protein that catalyzes debranching of lariat-intron RNAs. Transfection experiments demonstrate that hDbr1 is localized in a nucleoplasm of HeLa cells through a bipartite type nuclear localization signal near carboxyl-terminus. The conserved GNHE motif, originally identified in protein phosphatase protein family, is critical for hDbr1 to dissolve lariat structure in vitro. Furthermore, heterokaryon experiments show that hDbr1 is a nucleocytoplasmic shuttling protein, suggesting novel role(s) of hDbr1 in the cytoplasm.

1-4) Identification of a novel component C2ORF3 in the lariat-intron complex

To identify novel factors involved in the post-splicing intron turnover pathway, we

performed immunoprecipitation with known post-splicing factors, hPrp43 and TFIP11. As an interacting factor, we identified C2ORF3 protein by mass spectrometry. We found that C2ORF3 protein is present in the previously characterized Intron Large (IL) complex with an excised lariat intron. *In vitro* splicing using C2ORF3-depleted nuclear extracts showed significant repression of splicing, suggesting that C2ORF3 protein is required for pre-mRNA splicing through its presumable role in efficient intron turnover. Interestingly, C2ORF3 protein is localized in both the nucleoplasm and nucleoli, which suggests a potential function in rRNA processing.

2) rRNA quality control mechanisms:

How the eukaryotic cells deal with non-functional RNA molecules that were either mutated or damaged? We are searching for novel RNA quality control mechanisms in mammalian and yeast cells by mainly focusing on ribosomal RNAs.

Quality control mechanisms operate in various steps of ribosomal biogenesis to ensure the production of functional ribosome particles. It was previously reported that mature ribosome particles containing nonfunctional mutant rRNAs are also recognized and selectively removed by a cellular quality control system (nonfunctional rRNA decay; NRD). Here, we show that the NRD of 25S rRNA requires a ubiquitin E3 ligase component Rtt101p and its associated protein Mms1p, previously identified as factors involved in DNA repair. We revealed that a group of proteins associated with nonfunctional ribosome particles are ubiquitinated in a Rtt101-Mms1-dependent manner. 25S NRD was disrupted when ubiquitination was inhibited by the overexpression of modified ubiquitin molecules, demonstrating a direct role for ubiquitin in this pathway. These results uncovered an unexpected connection between DNA repair and the quality control of rRNAs. Our findings support a model in which responses to DNA and rRNA damages are triggerd by a common ubiquitin ligase complex, during genotoxic stress harmful to both molecules.

Although we have clarified that 25S NRD requires an E3 ubiquitin ligase complex, which is involved in ribosomal ubiquitination, the degradation process of nonfunctional ribosomes remained unknown. Using genetic screening, we further identified two ubiquitin-binding complexes, the Cdc48–Npl4–Ufd1 complex (Cdc48 complex) and the proteasome, as the factors involved in 25S NRD. We show that the nonfunctional 60S subunit is dissociated from the 40S subunit in a Cdc48 complex-dependent manner, before it is attacked by the proteasome. When we examined the nonfunctional 60S subunits that accumulated under proteasome-depleted conditions, the majority of mutant 25S rRNAs retained their full length at a single-nucleotide resolution. This indicates that the proteasome is an essential factor triggering rRNA degradation. We further showed that ribosomal ubiquitination can be stimulated solely by the suppression of the proteasome, suggesting that ubiquitin–proteasome-dependent RNA degradation occurs in broader situations, including in general rRNA turnover.

List of Publications

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DEPARTMENT OF BIOLOGICAL RESPONSES
LABORATORY OF BIOLOGICAL PROTECTION

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Professor	**Koichi Ikuta**
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Introduction

Our laboratory has made two major achievements. First, we have found that fetal and adult hematopoietic stem cells have different developmental potential to differentiate into lymphocytes. Second, we have demonstrated that interleukin-7 (IL-7) controls DNA recombination of lymphocyte antigen receptor genes by changing chromatin structure. Both of them are related with fundamental questions in medicine and biology.

Based on these findings, we are now pursuing research on development and regulation of the immune system, focusing on the following questions: (1) function of IL-7 receptor (IL-7R) in immune system; (2) control mechanism of lymphocyte antigen receptor genes by IL-7; (3) regulation of immune response by IL-7R expression; and (4) distribution and function of IL-7-producing cells in lymphoid organs.

Topics

The interleukin-7 receptor controls development and maturation of late stages of thymocyte subpopulations: S. TANI-ICHI, A. SHIMBA, K. WAGATSUMA, H. MIYACHI[1], S. KITANO[1], K. IMAI, T. HARA and K. IKUTA ([1]Reproductive Engineering Team, Institute for Virus Research, Kyoto University**)**

IL-7 is a cytokine essential for T lymphocyte development and homeostasis. However, little is known about the roles of IL-7 receptor α-chain (IL-7Rα) in late stages of T cell development. To address this question, we established IL-7Rα-floxed mice and crossed them with CD4-Cre transgenic mice. Resultant IL-7R conditional knockout (IL-7RcKO) mice exhibited

marked reduction in CD8 single positive (SP) T cells, regulatory T cells (Tregs) and natural killer T (NKT) cells in thymus. The proportion and proliferation of both mature CD4SP and CD8SP thymocytes were decreased without affecting Runx expression. In addition, expression of the glucocorticoid-induced TNF receptor (GITR) was reduced in CD4SP and CD8SP thymocytes, and expression of CD5 was decreased in CD8SP thymocytes. IL-7RcKO mice also showed impaired Treg and NKT cell proliferation and inhibition of NKT cell maturation. Bcl-2 expression was reduced in CD4SP and CD8SP thymocytes but not in Tregs and NKT cells, and introduction of a Bcl-2 transgene rescued frequency and CD5 expression of CD8SP thymocytes. Furthermore, IL-7RcKO mice exhibited greatly increased numbers of B cells and, to a lesser extent, of γδ T and dendritic cells in thymus. Overall, this study demonstrates that IL-7Rα differentially controls development and maturation of thymocyte subpopulations in late developmental stages and suggests that IL-7R expression on αβ T cells suppresses development of other cell lineages in thymus.

Lymphocyte-stromal cell interaction induces IL-7 expression by interferon regulatory factors: M. SEKAI, S. TANI-ICHI, M. YONEYAMA[1], T. FUJITA[1], T. KINA[2], and K. IKUTA ([1]Laboratory of Molecular Genetics, Institute for Virus Research, Kyoto University, [2]Department of Immunology, Institute for Frontier Medical Sciences, Kyoto University**)**

The interaction between lymphocytes and stromal cells plays important roles in coordinated development of early lymphocytes. IL-7 is an essential cytokine for early lymphocyte development produced by stromal cells in the thymus and bone marrow. Although IL-7 is induced by interaction of early lymphocytes and stromal cells, its molecular basis is still unknown. To address this question, we employed co-culture system with an IL-7-dependent pre-B cell line, DW34, and a thymic stromal cell line, TSt-4. Co-culture with DW34 cells enhanced the levels of IL-7 transcripts in TSt-4 cells. Interestingly, the co-culture also induced transcripts of IFN-α and IFN-β but not of IFN-γ. In addition, exogenous IFN-β stimulation increased the levels of IL-7 transcripts in TSt-4 cells. Next, to elucidate the molecular mechanism of IL-7 induction, we analyzed the IL-7 promoter activity by reporter assay. The IL-7 promoter showed specific transcriptional activity in TSt-4 cells. An interferon-stimulated response element (ISRE) in the IL-7 promoter was essential for the induction of IL-7 transcription by both co-culture and IFN-β stimulation. Finally, overexpression of wild-type and dominant-negative forms of interferon regulatory factors (IRFs) activated and repressed, respectively, the IL-7 promoter in TSt-4 cells. Collectively, these results suggested that IRFs activated by lymphocyte adhesion induce IL-7 transcription through ISRE in stromal cells and that type I IFNs may be involved in the activation

of IRFs. Thus, this study implied a physiological function of the IFN/IRF signal during lymphocyte development.

Interleukin-7 produced by thymic epithelial cells plays a major role in the development of thymocytes and TCR$\gamma\delta^+$ intraepithelial lymphocytes: S. SHITARA, T. HARA, B. LIANG, K. WAGATSUMA, S. ZUKLYS[1], G. A. HOLLÄNDER[1], H. NAKASE[2], T. CHIBA[2], S. TANI-ICHI and K. IKUTA ([1]Pediatric Immunology, Center for Biomedicine and the University Children's Hospital of Basel, [2]Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University)

IL-7 is a cytokine essential for T cell development and survival. However, the local function of IL-7 produced by thymic epithelial cells (TECs) is poorly understood. To address this question, we generated IL-7-floxed mice and crossed them with FoxN1-Cre mice to establish knockout mice conditionally deficient for the expression of IL-7 by TECs. We found that $\alpha\beta$ and $\gamma\delta$ T cells were significantly reduced in the thymus of IL-$7^{f/f}$ FoxN1-Cre mice. Proportion of mature single positive thymocytes was increased. In lymph nodes and spleen, the numbers of T cells were partially restored in IL-$7^{f/f}$ FoxN1-Cre mice. In addition, $\gamma\delta$ T cells were absent from fetal thymus and epidermis of IL-$7^{f/f}$ FoxN1-Cre mice. Furthermore, TCR$\gamma\delta^+$ intraepithelial lymphocytes (IELs) were significantly decreased in the small intestine of IL-$7^{f/f}$ FoxN1-Cre mice. To evaluate the function of IL-7 produced in the intestine, we crossed the IL-$7^{f/f}$ mice with villin-Cre mice to obtain the mice deficient in IL-7 production from intestinal epithelial cells. We observed that $\alpha\beta$ and $\gamma\delta$ IELs of IL-$7^{f/f}$ villin-Cre mice were comparable to control mice. Collectively, our results suggest that TEC-derived IL-7 plays a major role in proliferation, survival and maturation of thymocytes and is indispensable for $\gamma\delta$ T cell development. This study also demonstrates that IL-7 produced in the thymus is essential for the development of $\gamma\delta$ IELs and indicates the thymic origin of $\gamma\delta$ IELs.

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Sekai, M., Tani-ichi, S., Yoneyama, M., Fujita, T., Kina, T., and Ikuta, K. (2013). Lymphocyte-stromal cell interaction induces IL-7 expression by interferon regulatory factors. Mol. Immunol. *54*, 378-385.

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Wagatsuma, K., Tani-ichi, S., Liang, B., Shitara, S., Miyachi, H., Kitano, S., Kimura, H., Hara, T., and Ikuta, K.: STAT5 motifs in the T cell receptor Jγ promoters control the local chromatin accessibility and rearrangements of the Jγ gene segments. The 11th International Student Seminar, Kyoto, 5-6 March 2013.

Shitara, S., Hara, T., Liang, B., Wagatsuma, K., Tani-ichi, S., and Ikuta, K.: Interleukin-7 produced by thymic epithelial cells plays a major role in the development of thymocytes and TCR$\gamma\delta^+$ intraepithelial lymphocytes. The 11th International Student Seminar, Kyoto, 5-6 March 2013.

Ikuta, K., Cui, G., Shitara, S., Liang, B., Tani-ichi, S., and Hara, T.: Visualization of the immune microenvironment by reporter mice. The 6th International Workshop of Kyoto T Cell Conference 2013, Kyoto, 7 June 2013.

Hara, T., Cui, G., Shitara, S., Tani-ichi, S. and Ikuta, K.: Visualization and characterization of IL-7- and IL-15-expressing cells in vivo. The 6th International Workshop of Kyoto T Cell Conference 2013, Kyoto, 4-5 June 2013.

Wagatsuma, K., Tani-ichi, S., Shimba, A., Liang, B., Shitara, S., Hara, T., and Ikuta, K.: Differential roles of Jγ promoters and Eγ enhancers in controlling chromatin accessibility of the TCRγ locus by STAT5. The 6th International Workshop of Kyoto T Cell Conference 2013, Kyoto, 4-5 June 2013.

Hara, T. and Ikuta, K.: Distribution and function of IL-7-expressing cells in the intestinal tissues. The 8th International Symposium of the Institute Network, Kyoto, 27 June 2013.

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DEPARTMENT OF BIOLOGICAL RESPONSES
LABORATORY OF INFECTION AND PREVENTION

I. First Group

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Introduction

The research projects carried out in this group are aiming to uncover the molecular mechanisms of the regulation of inflammation in innate immunity. Since inflammation is mediated by the production of proinflammatory cytokines, we are studying the cytokine gene expression at the transcriptional and posttranscriptional levels.

Topics

Regulatory mechanism for the Regnase-1-mediated mRNA decay in innate immunity and its post-transcriptional regulation: T. MINO, A. FUKAO[1], T. IMAMURA, M. HARUNA, T. UEHATA, M. YOSHINAGA, T. FUJIWARA[1] and O. TAKEUCHI
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Gene expression in response to inflammatory stimuli is controlled at the transcriptional and post-transcriptional mechanisms in immune cells. Post-transcriptional regulation that modifies

mRNA stability and translation provides rapid and flexible control of gene expression. Control of mRNA stability is mediated by a set of RNA binding proteins including Tristetraprolin, Roquin and Regnase-1. Roquin (RING finger and CCCH zinc finger protein) prevents development of autoimmunity in mice by destabilizing the mRNA such as *inducible T cell costimulator (Icos)* and *tumor necrosis factor (Tnf)*. We have previously identified an RNase Regnase-1 (also known as Zc3h12a, Mcpip1) degrades mRNAs such as *Interleukin-6 (Il6*) and *Regnase-1* itself, and plays a critical role in preventing autoimmunity in mice. In the present study, we demonstrate that Regnase-1 is essential for preventing aberrant effector CD4+ T cell generation cell-autonomously by generating Regnase-1 conditional allele. Moreover, in T cells, Regnase-1 regulates the mRNAs of a set of genes, including c-Rel, Ox40 and Il2, through cleavage of their 3′-UTRs. Interestingly, T cell receptor (TCR) stimulation leads to cleavage of Regnase-1 at R111 by Malt1/paracaspase, freeing T cells from Regnase-1-mediated suppression. We found that Malt1 protease activity is critical for controlling mRNA stability of these genes. Taken together, these results indicate that dynamic control of Regnase-1 expression in T cells is critical for controlling T cell activation.

Furthermore, we investigated Regnase-1 target mRNA structures, and found that stem-loop motives present in immune-related mRNAs are shared by Regnase-1 and Roquin for recognition by RNA-immunoprecipitation sequencing in HeLa cells. Whereas Roquin localizes to stress granules (SGs) and processing bodies (PBs), which is cytoplasmic foci now known to be involved in the posttranscriptional processing of mRNAs, Regnase-1 localizes to cytoplasm and endoplasmic reticulum (ER), but not to PBs and SGs, and colocalizes with ribosome. Regnase-1-mediated target mRNA cleavage requires active transcription of the RNAs. Knockdown of Regnase-1 increases mRNA localized on polysome, whereas knockdown of Roquin increased mRNA localized on ribonucleoprotein complex (RNPs), suggesting that Regnase-1 and Roquin destabilizes translationally active and inactive mRNA, respectively. Regnase-1 is physically associated with the ribosome and translation termination is required for the Regnase-1-mediated mRNA decay. Thus, Regnase-1 and Roquin proteins act as spatial regulators of mRNA stability by recognizing a common stem-loop RNA degradation motifs.

Role of Akirin2 in Toll-like receptor-mediated cytokine gene expression in macrophages:

S. TARTEY, D. ORI, A. WAKABAYASHI, M. ABE, H. WAKABAYASHI and O. TAKEUCHI

The Toll-like receptor (TLR) signaling leads to the activation of a set of transcription factors including NF-κB and AP-1 for inducing proinflammatory cytokine genes. In addition, transcription of inflammatory genes in innate immune cells is coordinately regulated by transcription factors and chromatin modifiers. However, it remains unclear how microbial sensing

initiates chromatin remodeling. Here we show that Akirin2, an evolutionarily conserved nuclear protein, bridges NF-κB and the chromatin remodeling SWI/SNF complex by interacting with BRG1 Associated Factor 60 (BAF60) proteins as well as IκB-ζ, which forms a complex with the NF-κBp50 subunit. These interactions are essential for Toll-like receptor-, RIG-I- and Listeria-mediated expression of proinflammatory genes including Il6 and Il12b in macrophages. Consistently, effective clearance of Listeria infection required Akirin2. Akirin2 (IκB-ζ) recruitment to the Il6 promoter upon LPS stimulation was found to depend on IκB-ζ (Akirin2), where it regulates chromatin remodeling. BAF60 proteins were also essential for the induction of Il6 in response to LPS stimulation. Collectively, the IκB-ζ-Akirin2-BAF60 complex physically links the NF-κB and SWI/SNF complexes in innate immune cell activation.

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Uehata, T., Iwasaki, H., Vandenbon, Matsushita, K., Cuellar, E.H., Kuniyoshi, K., Satoh, T., Mino, T., Suzuki, Y., Standley, D.M., Tsujimura, T., Rakugi, H., Isaka, Y., Takeuchi, O., and Akira, S. (2013). Malt1-Induced Cleavage of Regnase-1 in CD4+ Helper T Cells Regulates Immune Activation. Cell *153*, 1036-1049.

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Ma, J., Bang, B.R., Lu, J., Eun, S.Y., Otsuka, M., Croft, M., Tobias, P., Han, J., Takeuchi, O., Akira, S., Karin, M., Yagita, H., and Kang, Y.J. (2013). The TNF family member 4-1BBL sustains inflammation by interacting with TLR signaling components during late-phase activation. Sci. Signal. *6*, ra87.

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Takeuchi, O.: Posttranscriptional control of inflammation by an RNase, Regnase-1. France - Japan Joint Forum “Frontiers in Innate Immunity” Strasbourg, France, 5-8 June 2013.

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竹内 理：Regnase-1 による炎症制御機構の解析、第 36 回日本分子生物学会年会、神戸市、2013 年 12 月 3 日

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Takeuchi, O.: Posttranscriptional regulation in chronic inflammation Annual Meeting of Japanese Society for Immunology. Makuhari, 11-13 December 2013.

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Ori, D., Kato, H., Sanjo, H., Tartey, S., Mino, T., Akira, S., and Takeuchi, O.: Essential roles of K63-linked polyubiquitin binding proteins, TAB2 and TAB3, in B cell activation via MAPKs. 11th International Student Seminar, Kyoto, Japan, 4-9 March 2013.

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Ori, D., Kato, H., Sanjo, H., Tartey, S., Mino, T., Akira, S., and Takeuchi, O.: Essential Roles of K63-Linked Polyubiquitin-Binding Proteins, TAB2 and TAB3 in B Cell Activation via MAPKs. The 12th Awaji International Forum on Infection and Immunity, No. P-79 (S5-6), Awaji, Japan. 10-13 September 2013.

Tartey, S., Akira, S., and Takeuchi, O.: Akirin2 is essential for inducing inflammatory genes in macrophages by bridging IκB-ζ and the chromatin remodeling complex. 20th East Asia Joint Symposium, Global Medical Science for the 22nd Century, Tokyo, Japan, 6-8 November 2013.

Tartey, S., Akira, S., and Takeuchi, O.: Akirin2 is indispensable for T cell development and maintenance of T cell homeostasis. 42nd Annual Meeting of the Japanese Society for Immunology, Chiba, Japan, 11-13 December 2013.

Uehata, T., Takeuchi, O., and Akira, S.: Regnase-1 regulates TCR-mediated T cell activation. 42nd Annual Meeting of the Japanese Society for Immunology, Chiba, Japan, 11-13 December 2013.

II. Second Group

Members

Associate Professor	**Hiroshi Masutani**
Research Student	**Cristiane Lumi Hirata**

Introduction

The research projects carried out in this group are studies on α-arrestin family proteins including thioredoxin binding protein-2 (TBP-2) also referred as thioredoxin interacting protein (Txnip) or Vitamin D3 up-regulated protein 1 (VDUP1). TBP-2 has attracted much attention as a multifunctional regulator in cancer suppression, metabolism, vascular stress, as well as immune response and inflammation. TBP-2 also acts as a negative regulator of thioredoxin. The other subject is redox signaling and host defense mechanism against oxidative stress. Thioredoxin is a key component of redox regulation and plays a protective role in various diseases, associated with oxidative stress and inflammation. In collaboration with Okayama University, thioredoxin has been reported to ameliorate influenza virus-induced acute lung injury in mice. In collaboration with Tohoku University, thioredoxin has been reported to attenuate early graft loss after intraportal islet transplantation in mice. Meanwhile, a member of the system in endoplasmic reticulum, Transmembrane Thioredoxin-Related Protein (TMX) has been reported to play an important protective role against inflammatory liver injury.

Topics

Development of redox modulating approaches against cancer and diabetes by Thioredoxin binding protein-2 (TBP-2)/Txnip: H. MASUTANI and C. L. HIRATA

TBP-2/Txnip$^{-/-}$ mice are much more susceptible to carcinogenesis than wild-type mice, indicating a role for TBP-2 in cancer suppression. TBP-2 regulates TGF-beta signaling and Epithelial-Mesenchymal transition. We here further investigated the mechanism by analyzing the interaction with NEDD family ubiquitin ligases such as Smurf1/2 and proteomics approaches. Meanwhile, TBP-2/Txnip plays essential roles in metabolic energy control. We previously showed that TBP-2/Txnip is a critical molecule in fasting response and that disruption of TBP-2/Txnip dramatically improves hyperglycemia, by enhanced insulin sensitivity with activated IRS-1/Akt signaling in skeletal muscle and augmented glucose-induced insulin secretion in beta cells. Based on the double face nature of TBP-2/Txnip, approaches against cancer and metabolic disorders are to

be designed. We showed that several reagents cause the change of TBP-2/Txnip expression, revealing novel aspects of the role of TBP-2/Txnip in biostress responses.

List of Publications

Asami, K., Inagaki, A., Imura, T., Sekiguchi, S., Fujimori, K., Masutani, H., Yodoi, J., Satomi, S., Ohuchi, N., and Goto, M. (2013). Thioredoxin-1 attenuates early graft loss after intraportal islet transplantation in mice. PLoS One *8*, e70259.

Matsuo, Y., Irie, K., Kiyonari, H., Okuyama, H., Nakamura, H., Son, A., Lopez-Ramos, D.A., Tian, H., Oka, S., Okawa, K., Kizaka-Kondoh, S., Masutani, H., and Yodoi, J. (2013). The protective role of the transmembrane thioredoxin-related protein TMX in inflammatory liver injury. Antioxid Redox Signal *18*, 1263-1272.

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DEPARTMENT OF CELL BIOLOGY
LABORATORY OF SUBCELLULAR BIOGENESIS

Members

Professor	**Fumiko Toyoshima**
Assistant Professor	**Shigeru Matsumura**
Lab Manager	**Nami Kohashi**
	Yoko Ohta
Graduate Student	**Mayumi Hamasaki**
	Keisuke Ikawa
	Sayaka Iwano
	Riki Ishibashi
	Megumi Ikeda
	Ryo Ichijo
	Mitsuko Fukuhara
	Kanae Mori
	Saori Yoneda

Introduction

Oriented cell division plays an essential role in asymmetric cell division, tissue morphogenesis and organogenesis. Our group seeks to explore the molecular mechanisms underlying the determination of cell division axis in culture cells and in mouse tissues. Our research is focused on the following subjects:

1, Mechanisms for orientated cell division in culture cells and skin basal cells.

2, Symmetric and asymmetric cell division of ES cells.

3, Metabolism and cell division.

Topics

Inhibition of endocytic vesicle fusion during mitosis: K. IKAWA, A. SATOU, M. FUKUHARA, S. MATSUMURA, N. SUGIYAMA, H. GOTO, M. FUKUDA, M. INAGAKI, Y. ISHIHAMA, F. TOYOSHIMA

Endocytic vesicle fusion is inhibited during mitosis, but the molecular pathways that mediate the inhibition remain unclear. Here we uncovered an essential role of polo-like kinase 1

(Plk1) in this mechanism. Phosphoproteomic analysis revealed that Plk1 phosphorylates the intermediate filament protein vimentin on Ser459, which is dispensable for its filament formation, but is necessary for the inhibition of endocytic vesicle fusion in mitosis. Furthermore, this mechanism is required for integrin trafficking toward the cleavage furrow during cytokinesis. Our results thus identify a novel mechanism for fusion inhibition in mitosis and implicate its role in vesicle trafficking after anaphase onset.

List of Publications

Ikawa, K.,Satou, A., Fukuhara, A., Matsumura, S., Sugiyama, N., Goto, H., Fukuda, M.,Inagaki, M.,*Ishihama, Y., and Toyoshima, F. (2014). Inhibition of endocytic vesicle fusion byPlk1-mediated phosphorylation of vimentin during mitosis. Cell Cycle *13*, 126-137.

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豊島文子：細胞分裂軸を決めるシグナル伝達ネットワーク、愛知県がんセンター特別招聘セミナー、愛知、2013 年 3 月 14 日

Fumiko Toyoshima: Pregnenolone Associates with Mitotic Spindles and Functions in Centriole Cohesion. The 25th CDB Meeting “Cilia and Centrosomes: from Fertilization to Cancer” Kobe, 17-18 June, 2013.

Fumiko Toyoshima: A novel role of pregnenolone in centriol cohesion.　第 65 回日本細胞生物学会、愛知、2013 年 6 月 19-21 日

豊島文子:ステロイドによる細胞分裂制御、第 32 回分子病理学研究会「吉野シンポジウム」、奈良、2013 年 7 月 20-21 日

豊島文子：細胞分裂の軸回転と生き物の形づくり、IGRE グリーン自然科学レクチャー、名古屋、2013 年 7 月 26 日

豊島文子:　細胞分裂期の膜と骨格を制御する新たなリン酸化シグナル伝達、第 86 回日本生

化学会大会シンポジウム、横浜、2013 年 9 月 11-13 日

濱崎眞弓、松村 繁、豊島文子：プレグネノロンは中心小体エンゲージメントを制御する、第 65 回日本細胞生物学会、愛知、2013 年 6 月 19-21 日

松村 繁、豊島文子：間期細胞形態と細胞分裂軸方向を繋ぐシグナル伝達機構の解析、第 65 回日本細胞生物学会、愛知、2013 年 6 月 19-21 日

Sayaka Iwano, Shigeru Matsumura, Ayaka Satou, Masaki Wakabayashi,Yasushi Ishihama and Fumiko Toyoshima : PCTK1 Regulates Spindle Orientation via PKA Regulatory Subunit, KAP0 第 65 回日本細胞生物学会、愛知、2013 年 6 月 19-21 日

一條 遼、松村 繁、豊島文子：妊娠期における皮膚基底細胞の増殖制御機構の解析、第 65 回日本細胞生物学会、愛知、2013 年 6 月 19-21 日

池田 愛、前川桃子、豊島文子 : 分化誘導後に ES 細胞が未分化状態を維持する機構の解析、日本細胞生物学会、愛知、2013 年 6 月 19-21 日

Sayaka Iwano, Shigeru Matsumura, Ayaka Satou, Masaki Wakabayashi,Yasushi Ishihama and Fumiko Toyoshima: Characterization of PCTK1 as a Novel Regulator for Oriented Cell Division, 第 8 回研究所ネットワーク国際シンポジウム、京都、2013 年 6 月 27-28 日

井川敬介、佐藤綾香、松村 繁、杉山直幸、後藤英仁、福田光則、稲垣昌樹、石濱 泰、豊島文子：Plk1 は分裂期における vimentin のリン酸化を介して初期エンドソームの fusion を阻害する、第 86 回日本生化学会大会、横浜、2013 年 9 月 11-13 日

濱崎眞弓、松村 繁、豊島文子：Pregnenolone Associates with Mitotic Spindles and Functions in Centriole Cohesion、第 86 回日本生化学会大会、横浜、2013 年 9 月 11-13 日

石橋理基、前川桃子、豊島文子：マウス ES 細胞の中胚葉と内胚葉への分化制御機構の解析、第 36 回日本分子生物学会、神戸、2013 年 12 月 3-6 日

Sayaka Iwano, Shigeru Matsumura, Ayaka Satou, Masaki Wakabayashi,Yasushi Ishihama and Fumiko Toyoshima：PCTK1 Regulates Spindle Orientation by phosphorylating PKA Regulatory Subunit KAP0, 第 36 回日本分子生物学会、神戸、2013 年 12 月 3-6 日

一條 遼、松村 繁、豊島文子：妊娠期における皮膚基底細胞の増殖制御機構の解析、第 36 回日本分子生物学会、神戸、2013 年 12 月 3-6 日

池田 愛、前川桃子、豊島文子：分化環境下での多能性維持を促進する転写因子の探索、第 36 回日本分子生物学会、神戸、2013 年 12 月 3-6 日

DEPARTMENT OF CELL BIOLOGY
LABORATORY OF GROWTH REGULATION

Members

Professor	**Ryoichiro Kageyama**
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	Kumiko Kobayashi
	Kyogo Kawaguchi
	Yuki Maeda
	Anna Araki
	Chihiro Masumoto

Introduction

The research interest of this laboratory is to understand the molecular mechanism of cell differentiation and organogenesis. Particularly, we are interested in basic helix-loop-helix (bHLH) transcription factors that regulate various developmental processes including neural development and somite formation. We are characterizing the functions of bHLH genes by misexpressing the genes with virus vectors and electroporation (gain-of-function study) and by generating knock-out mice (loss-of-function study). We previously showed that bHLH proneural genes such as *Ascl1* (also called *Mash1*) and *Math3* promote neuronal versus glial fate determination, whereas the bHLH repressor genes *Hes1* and *Hes5* regulate maintenance of neural stem cells by repressing proneural gene expression. These results indicate that the balance between bHLH proneural and bHLH repressor genes is important for a choice favoring neuronal differentiation or neural stem cell proliferation. It was recently shown however that the proneural gene *Ascl1* not only promotes neuronal fate determination but also induces proliferation of neural stem cells. In addition, it was

found that the bHLH gene *Olig2* regulates both oligodendrocyte fate determination and neural stem cell proliferation. Our group found that Hes1 promotes astrocyte formation as well as neural stem cell proliferation. Thus, each bHLH fate determination factor has contradictory functions, neural stem cell proliferation and specific cell fate determination, but the detailed mechanism of how these bHLH factors regulate such contradictory functions is unknown.

We found that in neural stem cells, Hes1 expression oscillates by negative feedback with a period of about 2-3 hours. Hes1 oscillations drive cyclic expression of the proneural genes *Ascl1* and *Neurog2* and the Notch ligand gene *Deltalike1* (*Dll1*). In contrast, the expression of *Ascl1*, *Neurog2* and *Dll1* is sustained (non-oscillatory) in postmitotic differentiating neurons. We also found that although Hes1 expression oscillates in neural stem cells, it becomes up-regulated and sustained during astrocyte formation. Similarly, the bHLH factor Olig2 expression oscillates in neural stem cells but becomes up-regulated and sustained during oligodendrocyte formation. Our results therefore suggest that the multipotency is a state of oscillatory expression of multiple fate determination factors, while the fate determination is a process of dominant expression of a selected single bHLH factor, which represses the other fate determination factors. We further showed by a new optogenetics approach that sustained expression of the proneural gene *Ascl1* induces neuronal fate determination, whereas oscillatory expression of Ascl1 activates proliferation of neural stem cells. Thus, the expression dynamics are very important for a choice between neural stem cell proliferation and specific cell fate determination.

Topics

1) Oscillatory control of factors determining multipotency and fate in mouse neural progenitors: I. IMAYOSHI, A. ISOMURA, Y. HARIMA, K. KAWAGUCHI, H. KORI, H. MIYACHI, T. FUJIWARA, F. ISHIDATE and R. KAGEYAMA

The basic helix-loop-helix transcription factors Ascl1/Mash1, Hes1, and Olig2 regulate fate choice of neurons, astrocytes, and oligodendrocytes, respectively. These same factors are coexpressed by neural progenitor cells. Here, we found by time-lapse imaging that these factors are expressed in an oscillatory manner by mouse neural progenitor cells. In each differentiation lineage, one of the factors becomes dominant. We used optogenetics to control expression of Ascl1 and found that, although sustained Ascl1 expression promotes neuronal fate determination, oscillatory Ascl1 expression maintains proliferating neural progenitor cells. Thus, the multipotent state correlates with oscillatory expression of several fate-determination factors, whereas the differentiated state correlates with sustained expression of a single factor.

2) Hedgehog signaling regulates prosensory cell properties during the basal-to-apical wave of hair cell differentiation in the mammalian cochlea: T. TATEYA, I. IMAYOSHI, I. TATEYA, K. HAMAGUCHI, H. TORII, J. ITO and R. KAGEYAMA

Mechanosensory hair cells and supporting cells develop from common precursors located in the prosensory domain of the developing cochlear epithelium. Prosensory cell differentiation into hair cells or supporting cells proceeds from the basal to the apical region of the cochleae, but the mechanism and significance of this basal-to-apical wave of differentiation remain to be elucidated. Here, we investigated the role of Hedgehog (Hh) signaling in cochlear development by examining the effects of up- and downregulation of Hh signaling in vivo. The Hh effector smoothened (Smo) was genetically activated or inactivated specifically in the developing cochlear epithelium after prosensory domain formation. Cochleae expressing a constitutively active allele of Smo showed only one row of inner hair cells with no outer hair cells (OHCs); abnormal undifferentiated prosensory-like cells were present in the lateral compartment instead of OHCs and their adjacent supporting cells. This suggests that Hh signaling inhibits prosensory cell differentiation into hair cells or supporting cells and maintains their properties as prosensory cells. Conversely, in cochlea with the Smo conditional knockout (Smo CKO), hair cell differentiation was preferentially accelerated in the apical region. Smo CKO mice survived after birth, and exhibited hair cell disarrangement in the apical region, a decrease in hair cell number, and hearing impairment. These results indicate that Hh signaling delays hair cell and supporting cell differentiation in the apical region, which forms the basal-to-apical wave of development, and is required for the proper differentiation, arrangement and survival of hair cells and for hearing ability.

3) Control of Hes7 expression by Tbx6, the Wnt pathway and the chemical Gsk3 inhibitor LiCl in the mouse segmentation clock: A. GONZALEZ, I. MANOSALVA, T. LIU and R. KAGEYAMA

The mouse segmentation is established from somites, which are iteratively induced every two hours from the presomitic mesoderm (PSM) by a system known as the segmentation clock. A crucial component of the segmentation clock is the gene Hes7, which is regulated by the Notch and Fgf/Mapk pathways, but its relation to other pathways is unknown. In addition, chemical alteration of the Wnt pathway changes the segmentation clock period but the mechanism is unclear.To clarify these questions, we have carried out Hes7 promoter analysis in transgenic mouse embryos and have identified an essential 400 bp region, which contains binding sites of Tbx6 and the Wnt signaling effector Lef1. We have found that the Hes7 promoter is activated by Tbx6, and normal activity of the Hes7 promoter in the mouse PSM requires Tbx6 binding sites. Our results demonstrate that Wnt pathway molecules activate the Hes7 promoter cooperatively with Tbx6 in cell culture and are

necessary for its proper expression in the mouse PSM. Furthermore, it is shown that the chemical Gsk3 inhibitor LiCl lengthens the oscillatory period of Hes7 promoter activity.Our data suggest that Tbx6 and the Wnt pathway cooperatively regulate proper Hes7 expression. Furthermore, proper Hes7 promoter activity and expression is important for the normal pace of oscillation.

4) Hes1 in the somatic cells of the murine ovary is necessary for oocyte survival and maturation: I. MANOSALVA, A. GONZALEZ and R. KAGEYAMA

The Notch pathway plays an important role in ovary development in invertebrates like Drosophila. However its role for the mammalian ovary is unclear. Mammalian Hes genes encode transcriptional factors that mediate many of the activities of the Notch pathway. Here, we have studied the function of Hes1 during embryonic development of the mouse ovary. We find that Hes1 protein is present in somatic cells and oocyte cytoplasm and decreases between E15.5 and P0. Conventional Hes1 knock-out (KO), Hes1 conditional KO in the ovarian somatic, and chemical inhibition of Notch signaling decrease the total number, size and maturation of oocytes and increase the number of pregranulosa cells at P0. These defects correlate with abnormal proliferation and enhanced apoptosis. Expression of the proapoptotic gene Inhbb is increased, while the levels of the antiapoptotic and oocyte maturation marker Kit are decreased in the Hes1 KO ovaries. Conversely, overactivation of the Notch pathway in ovarian somatic cells increases the number of mature oocytes and decreases the number of pregranulosa cells. Fertility is also reduced by either Hes1 deletion or Notch pathway overactivation. In conclusion, our data suggest that the Notch-Hes1 pathway regulates ovarian somatic cell development, which is necessary for oocyte survival and maturation.

5) Oscillatory links of Fgf signaling and Hes7 in the segmentation clock: Y. HARIMA and R. KAGEYAMA

Somitogenesis is controlled by the segmentation clock, where the oscillatory expression of cyclic genes such as Hes7 leads to the periodic expression of Mesp2, a master gene for somite formation. Fgf signaling induces the oscillatory expression of Hes7 while Hes7 drives coupled oscillations in Fgf and Notch signaling, which inhibits and activates Mesp2 expression, respectively. Because of different oscillatory dynamics, oscillation in Fgf signaling dissociates from oscillation in Notch signaling in S-1, a prospective somite region, where Notch signaling induces Mesp2 expression when Fgf signaling becomes off. Thus, oscillation in Fgf signaling regulates the timing of Mesp2 expression and the pace of somitogenesis. In addition, Fgf signaling was found to be a primary target for hypoxia, which causes phenotypic variations of heterozygous mutations in Hes7 or Mesp2, suggesting gene-environment interaction through this signaling.

List of Publications

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下條博美、播磨有希子、前田勇樹、大塚俊之、宮地 均、影山龍一郎：神経発生過程における遺伝子発現ダイナミクスによる神経分化制御機構の解明、第 36 回日本分子生物学会年会、神戸、2013 年 12 月 3-6 日

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DEPARTMENT OF CELL BIOLOGY
LABORATORY OF SIGNAL TRANSDUCTION

Members

Associate Professor Takayuki Miyazawa

Introduction

Our research objective is to understand the pathogenesis of animal retroviruses, functions of endogenous retroviruses and potential iatrogenic risks by infection of endogenous retroviruses in xenotransplantation and vaccination. We are currently studying simian retroviruses, feline endogenous retroviruses, bovine endogenous retroviruses and koala retroviruses.

Topics

1) Fematrin-1 is involved in fetomaternal cell-to-cell fusion in Bovinae placenta and has contributed to diversity of ruminant placentation: Y. NAKAYA, K. KOSHI, S. NAKAGAWA, K. HASHIZUME and T. MIYAZAWA

During placentation, mammals employ different strategies for nourishing and supporting fetuses. Members of the Bovidae family, consisting of cloven-hoofed ruminants, utilize multiple maternal attachment points on the placenta, known as cotyledons, and hybrid cells, named trinucleate cells or syncytial plaques, made up of a fusion of fetal trophoblasts and maternal endometrial cells to provide essential hormones and maintain long gestation periods. These hybrid cells are unique to the Bovidae, as fetomaternal borders are clearly separated by syncytiotrophoblasts or epithelial cells in the placenta of other mammals. Recently, it was reported that Syncytin-Rum1 was inserted into ruminant genomes, including cattle and sheep, and was possibly involved in fetomaternal cell-to-cell fusion in both species. However, Syncytin-Rum1 alone is insufficient to explain the morphological diversity of the fetomaternal hybrids between Bovinae and Caprinae (i.e., trinucleate cells in Bovinae and syncytial plaques in Caprinae). Here we report that the bovine endogenous retrovirus K1 (BERV-K1) envelope, which we term Fematrin-1, was specifically expressed in binucleated trophoblasts throughout gestation in cattle and induced fusion with bovine endometrial cells in vitro at a significantly higher level than Syncytin-Rum1 under physiological conditions. Fematrin-1 was found to be integrated into intron 18 of FAT tumor suppressor homolog 2 (FAT2) about 18.3 to 25.4 million years ago and has been subject to purifying selection through the evolution of Bovinae. Phylogenetically, Fematrin-1 is distinct from Syncytin

genes found in other mammalian species that form syncytiotrophoblasts. Our results suggest that the newly acquired endogenous retroelement has contributed to generating placentation diversity through ruminant evolution.

2) Identification of a novel subgroup of Koala retrovirus from Koalas in Japanese zoos : T. SHOJIMA, R. YOSHIKAWA, S. HOSHINO, S. SHIMODE, S. NAKAGAWA, T. OHATA, R. NAKAOKA, and T. MIYAZAWA.

We identified a new subgroup of koala retrovirus (KoRV), named KoRV-J, which utilizes thiamine transport protein 1 as a receptor instead of the Pit-1 receptor used by KoRV (KoRV-A). By subgroup-specific PCR, KoRV-J and KoRV-A were detected in 67.5 and 100% of koalas originating from koalas from northern Australia, respectively. Altogether, our results indicate that the invasion of the koala population by KoRV-J may have occurred more recently than invasion by KoRV-A. KoRV-J isolate (strain OJ-4) has three 37bp tandem repeats named DR-2. We also found that the promoter activity of the KoRV-J strain OJ-4 is stronger than that of original KoRV-A, suggesting that KoRV-J may replicate more efficiently than KoRV-A.

3) Construction and characterization of an infectious molecular clone of Koala retrovirus : T. SHOJIMA, S. HOSHINO, M. ABE, J. YASUDA, H. SHOGEN, T. KOBAYASHI, and T. MIYAZAWA

Koala retrovirus (KoRV) is a gammaretrovirus that is currently endogenizing into koalas. Studies on KoRV infection have been hampered by the lack of a replication-competent molecular clone. In this study, we constructed an infectious molecular clone, termed plasmid pKoRV522, of a KoRV isolate (strain Aki) from a koala reared in a Japanese zoo. The virus KoRV522, derived from pKoRV522, grew efficiently in human embryonic kidney (HEK293T) cells, attaining 10(6) focus-forming units/ml. Several mutations in the Gag (L domain) and Env regions reported to be involved in reduction in viral infection/production in vitro are found in pKoRV522, yet KoRV522 replicated well, suggesting that any effects of these mutations are limited. Indeed, a reporter virus pseudotyped with pKoRV522 Env was found to infect human, feline, and mink cell lines efficiently. Analyses of KoRV L-domain mutants showed that an additional PPXY sequence, PPPY, in Gag plays a critical role in KoRV budding. it was demonstrated that WWP2 or WWP2-like E3 ubiquitin ligases possessing the WW domain closely related to WWP2 and Vps4A/B are involved in KoRV budding. These data suggest that KoRV Gag recruits the cellular endosomal sorting complex required for transport machinery through interaction of the PPPY L-domain with the WW domain(s) of WWP2 and that progeny virions are released from cells by utilizing the multivesicular body sorting pathway. Altogether, our results demonstrate the construction and characterization of

the first infectious molecular clone of KoRV. The infectious clone reported here will be useful for elucidating the mechanism of endogenization of the virus in koalas and screening for antiretroviral drugs for KoRV-infected koalas.

4) Characterization of feline ASCT1 and ASCT2 as RD-114 virus receptor : S. SHIMODE, R NAKAOKA, H. SHOGEN, and T. MIYAZAWA

RD-114 virus is a replication-competent feline endogenous retrovirus (ERV). RD-114 virus had been thought to be xenotropic; however, recent findings indicate that RD-114 virus is polytropic and can infect and grow efficiently in feline cells. Receptor(s) for RD-114 virus has not been identified and characterized in cats. In this study, we confirmed that two feline sodium-dependent neutral amino acid transporters (ASCTs), fASCT1 and fASCT2, function as RD-114 virus receptors. By chimeric analyses of feline and murine ASCTs, we revealed that extracellular loop 2 of both fASCT1 and fASCT2 determines the susceptibility to RD-114 virus. Further, we revealed ubiquitous expression of these genes, consistent with the general metabolic role of the ASCT molecules. Our study indicates that RD-114 virus may reinfect tissues and cells in cats, once the virus is activated. Implications of the involvement of RD-114 virus in feline oncogenesis are also discussed.

5) A new approach to establish a cell line with reduced risk of endogenous retroviruses : A. FUKUMA, R. YOSHIKAWA, T. MIYAZAWA and J. YASUDA

Endogenous retroviruses (ERVs) are integrated as DNA proviruses in the genomes of all mammalian species. Several ERVs are replication-competent and produced as fully infectious viruses from host cell. Thus, live-attenuated vaccines and biological substances have been prepared using the cell lines which may produce ERV. Indeed, we recently reported that several commercial live-attenuated vaccines for pets were contaminated with the infectious feline endogenous retrovirus, RD-114. In this study, to establish a cell line for vaccine manufacture with reduced risk of ERVs, we generated a cell line stably expressing human tetherin (Teth-CRFK cells). The release of infectious ERV from Teth-CRFK cells was suppressed to undetectable levels, while the production of parvovirus in Teth-CRFK cells was similar to that in parental CRFK cells. These observations suggest that Teth-CRFK cells will be useful as a cell line for the manufacture of live-attenuated vaccines or biological substances with reduced risk of ERV.

List of Publications

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宮沢孝幸*（2013）FIV 感染ネコへのエール : 君はきっと FIV に打ち克つ（前編）NJK 144、28-33.

宮沢孝幸*（2013）FIV 感染ネコへのエール : 君はきっと FIV に打ち克つ（後編）NJK 145、31-34.

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Nakaya, Y., Koshi, K., Nakagawa, S., Nakaya T., Hashizume, K., and Miyazawa, T.: Fematrin-1 contributed to generating the diversity of ruminant placentation. 25th Workshop on Retroviral Pathogenesis, Reykjavik, Iceland, 7-10 August 2013.

Yoshikawa, R., Okamoto, M., Miura, T., Hirai, H., and Miyazawa, T.: Identification of a causative agent of thrombocytopenia in Japanese monkeys (Macaca fuscata) as simian retrovirus type 4. 25th Workshop on Retroviral Pathogenesis, Reykjavik, Iceland, 7-10 August 2013.

Takayuki Miyazawa.: Exogenous and endogenous betaretroviruses; implications in the evolution of mammals. 1st Kyoto International Symposium on Virus-Host Coevolution , Kyoto, Japan, 7 November 2013.

吉川禄助、下出紗弓、中岡里江、坂口翔一、宮沢孝幸：ニホンザル由来サルフォーミーウイルスの全塩基配列決定と感染クローンの作製、第 155 回日本獣医学会学術集会、東京、2013 年 3 月 28-30 日

下出紗弓、吉川禄助、仲屋友喜、中岡里江、小林 剛、宮沢孝幸：ネコにおける RD-114 ウイルス受容体の同定および機能解析、第 155 回日本獣医学会学術集会、東京、2013 年 3 月 28-30 日

今川和彦、安田二朗、宮沢孝幸：生殖研究の新展開：ウシ受胎率の向上に向けて、第 155 回日本獣医学会学術集会、東京、2013 年 3 月 28-30 日

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佐藤英次、鈴木樹理、渡邉朗野、兼子明久、吉川禄助、吉田友教、山中淳史、齋藤 暁、齋藤波子、坂口翔一、明里宏文、宮沢孝幸、岡本宗裕：ニホンザルにおけるサルレトロウイルス 4 型の臓器特異性の調査、第 156 回日本獣医学会学術集会、岐阜、2013 年 9 月 20-22 日

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佐藤英次、兼子明久、齋藤 暁、山中淳史、鈴木樹理、渡邉朗野、吉田友教、吉川禄助、齋藤波子、牧野瀬恵美子、道家由美子、塩澤裕子、安江美雪、森本真弓、宮沢孝幸、明里宏文、岡本宗裕：サルレトロウイルス 4 型持続感染ニホンザルにおける免疫学的役割の解明、第 61 回日本ウイルス学会学術集会、神戸、2013 年 11 月 10-12 日

仲屋友喜、中屋隆明、宮沢孝幸：レトロウイルス RNA 核外輸送機構における内在化の影響、第 61 回日本ウイルス学会学術集会、神戸、2013 年 11 月 10-12 日

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宮沢孝幸：古代レトロウイルスとほ乳類の進化第 36 回日本分子生物学会年会、神戸、2013 年 12 月 3-6 日

CENTER FOR HUMAN RETROVIRUS RESEARCH
LABORATORY OF VIRAL PATHOGENESIS

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	Tomoko Kobayashi
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Introduction

We have been focusing on basic researches of human viruses, including human immunodeficiency virus type 1 (HIV-1), herpes simplex virus type 1 (HSV-1), and other human viruses. The goal of our researches is to elucidate the molecular mechanisms of viral pathogenesis and to find the strategy for treatment. We generated a humanized mouse model that NOG mice are transplanted with human heamatopoietic stem cells (HSCs). Using this model, we have a series of projects aimed at elucidating how accessory HIV-1 proteins (Vpr, Vif, and Vpu) contribute to viral replication and its pathogenesis *in vivo*. Viral replication was completely attenuated in humanized mice infected with *vif*-deficient condition (Sato *et al. JV* 2010). In contrast, profound attenuation of *vpr*-deficient HIV-1 replication was found preferentially in regulatory $CD4^+$ T cells (Tregs). We have other series of research projects by using cell-cultured base systems and reported novel observation. We generated a genome-editing strategy for HIV provirus using clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 system and found TAR region of HIV-1 LTR is a proper target. SAMHD1 is a potential innate anti-viral player that suppresses the replication of a wide range of DNA viruses including HSV-1, as observed in HIV, in non-dividing myeloid cells. We performed an experimental-mathematical investigation for replication property of enterovirus 71 (EV71) and also other human viruses.

Topics

HIV-1 infection in humanized mice: K. SATO, MISAWA and Y. KOYANAGI

The precise role of Vpr during HIV-1 infection and its contribution to the development of AIDS remain unclear. Previous reports have shown that Vpr has the ability to cause G2 cell cycle arrest and apoptosis in HIV-1-infected cells *in vitro*. In addition, *vpr* is highly conserved in transmitted/founder HIV-1s and in all primate lentiviruses, which are evolutionarily related to HIV-1. Although these findings suggest an important role of Vpr in HIV-1 pathogenesis, its direct evidence *in vivo* has not been shown. By using humanized mouse model, we demonstrated that Vpr causes G2 cell cycle arrest and apoptosis predominantly in proliferating $MKI67^+$ $CCR5^+$ $CD4^+$ T cells, which mainly consist of Tregs, resulting in Treg depletion and enhanced virus production during acute infection. The Vpr-dependent enhancement of virus replication and Treg depletion is observed infected with CCR5-tropic but not CXCR4-tropic HIV-1, suggesting that these effects are dependent on the coreceptor usage by HIV-1. Immune activation was observed in wild-type (WT) not in *vpr*-deficient HIV-1-infected humanized mice. When humanized mice were treated with denileukin diftitox (DD), to deplete Tregs, DD-treated humanized mice showed massive activation/proliferation of memory T cells compared to the untreated group. This activation/proliferation enhanced CCR5 expression in memory $CD4^+$ T cells and rendered them accelerating susceptible to HIV-1. These results suggest that Vpr takes advantage of proliferating $CCR5^+$ $CD4^+$ T cells for enhancing viremia of HIV-1. Because Tregs exist in a higher cycling state than other T cell subsets, Tregs appear to be more vulnerable to exploitation by Vpr during acute HIV-1 infection (Sato *et al*. *PLOS Pathog*., 2013).

HIV-1 evolution: K. SATO, J. S. TAKEUCHI, T. KOBAYASHI, Y. KIMURA, N. MISAWA and Y. KOYANAGI

Primate lentiviruses evolved through the acquisition of antagonists against intrinsic host restriction factors, such APOBEC3G and tetherin. We found that SIV Nef derived from old world monkey antagonizes own tetherin, suggesting that some SIV Nef share a common ancestor with other SIV Nef. More importantly, molecular phylogenetic analyses reveal that tetherins of some genus of old world monkeys are under positive selection, which is presumably accelerated by primate lentiviruses.

Genome-Editing for HIV cure: H. EBINA, N. MISAWA, Y. KANEMURA and Y. KOYANAGI

Latent infection occurs when the HIV-1 provirus becomes transcriptionally inactive, resulting in a latent reservoir that has become the main obstacle in preventing viral eradication from HIV-1 infected individuals. While highly active anti-retroviral therapy (HARRT) has dramatically decreased mortality from HIV-1 infection, there is currently no effective strategy to target the latent

form of HIV-1 proviruses. We validated the HIV-1 proviral-editing strategy using the latest genome-editing technology, CRISPR/Cas9 system. We have demonstrated the efficacy of the CRISPR/Cas9 system for HIV-1 provirus editing as follows. (1) The CRISPR/Cas9 system targeted for the TAR region of HIV-1 LTR drastically repressed HIV-1 expression. (2) The CRISPR/Cas9 system was available in T cells. (3) This HIV-1 LTR-targeting CRISPR/Cas9 system was strongly effective against latently integrated HIV-1 proviruses. (4) The CRISPR/Cas9 system targeting the LTR permitted excision of HIV-1 provirus (Ebina *et al*. *Sci. Rep.* 2013). We chose the TAR region of HIV LTR as the target of genome editing because the region is absolutely imperative for efficient elongation of HIV RNA. Therefore, the TAR region may be one of the best targets for HIV-1 proviral editing. Proviral editing can be an alternative strategy for HIV therapy for cure.

HSV-1 Restriction in Non-Dividing Myeloid cells: P. GEE, Y. KANEMURA, N. KASAI, H. EBINA and Y. KOYANAGI

Various viral pathogens such as HIV-1 and HSV-1 infect terminally-differentiated/non-dividing macrophages during the course of viral pathogenesis. Unlike dividing cells, non-dividing cells lack chromosomal DNA replication, do not enter the cell cycle, and harbor very low levels of cellular dNTPs, which are substrates of viral DNA polymerases. A series of recent studies revealed that the host protein SAMHD1 is dNTP triphosphohydrolase, which contributes to the poor dNTP abundance in non-dividing myeloid cells, and restricts proviral DNA synthesis of HIV-1 and other lentiviruses in macrophages, dendritic cells, and resting T cells. We found that SAMHD1 also controls the replication of large dsDNA viruses, HSV-1 and vaccinia virus (collaboration with Dr. Baek Kim), in primary human monocyte-derive dendritic cells (Hollenbaugh and Gee *et al*. *PLOS Pathog.* 2013). Our study suggests that SAMHD1 is a potential innate anti-viral player that suppresses the replication of a wide range of DNA viruses, as well as retroviruses, which infect non-dividing myeloid cells.

Experimental-Mathematical Investigation of Viruses: M. FUKUHARA, T. KOBAYASHI, J. S. TAKEUCHI, K. SATO, S. IWAMI and Y. KOYANAGI

We created a novel mathematical model of viral replication that is incorporated from experimental data and then found that virus productivity and transmissibility but not the cytotoxicity are drastically different among viral strains and can be associated with their epidemiological backgrounds in the case of EV71. The synergistic experimental-mathematical strategy is a powerful tool and will be used to quantitatively investigate the dynamics of virus infections not normally accessible by conventional experimental strategies (Fukuhara *et al*. *J. Virol.*, 2013). We also have another project of investigation of HIV-1 replication under the pressure of

APOBEC3G or APOBEC3F either deaminase-dependent or independent condition.

List of Publications

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金村優香、Peter Gee, 蝦名博貴、Yoo Ji Seung, 藤田尚志、小柳義夫： Novel function of SAMHD1 to involve in regulation of type I interferon induction. 第 36 回日本分子生物学会年会、

神戸、2013 年 12 月 4 日

CENTER FOR HUMAN RETROVIRUS RESEARCH
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Introduction

Both human T-cell leukemia virus type 1 (HTLV-1) and human immunodeficiency virus (HIV) are pathogenic human retroviruses. HTLV-1 promotes clonal proliferation of $CD4^+$ T cells, which leads to adult T-cell leukemia (ATL), while HIV decreases $CD4^+$ T cells resulting in onset of acquired immunodeficiency syndrome (AIDS). Our research objectives are to clarify the molecular mechanisms of virus-induced diseases, and to develop novel therapeutic strategies through research of these human retroviruses.

Topics

Roles of HTLV-1 bZIP factor (HBZ) in pathogenesis by HTLV-1: J. YASUNAGA, P. MIYAZATO, K. SUGATA, G. MA, Y. MITOBE, Y. MITAGAMI, H. KINOSADA and

M. MATSUOKA.

Human T-cell leukemia virus type 1 (HTLV-1) causes a neoplastic disease, adult T-cell leukemia (ATL), and inflammatory diseases. HTLV-1 encodes regulatory genes (*tax* and *rex*) and several accessory genes, including *p30, p12, p13* and *HTLV-1 bZIP factor* (*HBZ*). Tax and HBZ are thought to play important roles in HTLV-1-induced pathogenesis. Tax expression is frequently silenced in ATL cells while the *HBZ* gene transcription is detected in all of the ATL cell lines and primary ATL cases, indicating that HBZ is critical for ATL leukemogenesis. We have reported that HBZ-transgenic (Tg) mice develop T-cell lymphomas and systemic inflammatory diseases, such as dermatitis and alveolitis. This indicates that HBZ is closely linked with both T-cell lymphoma and inflammatory diseases. Immunological analyses showed that regulatory T cells (Tregs) were increased in HBZ-Tg. Interestingly, the suppressive function of Tregs from HBZ-Tg was impaired compared with non-Tg littermates, suggesting that HBZ expression increases dysfunctional Tregs resulting in chronic inflammation and malignant transformation *in vivo*. Recently, we have reported that HBZ enhances the generation of inducible Treg (iTreg) cells, which is likely converted to $Foxp3^-$ T cells producing IFN-γ, *in vivo*. HBZ-mediated proinflammatory phenotype of $CD4^+$ T cells is likely implicated in the pathogenesis of HTLV-1-associated inflammation. We are now investigating about the association between inflammation and oncogenesis induced by HBZ using this mouse model.

Molecular functions of HBZ in ATL leukemogenesis: A. TANAKA-NAKANISHI, G. MA, P. MIYAZATO, Y. MITOBE, N. SONO, K. YASUMA, A. KAWATSUKI, M. MOHAMED, R. FURUTA, J. YASUNAGA and M. MATSUOKA.

HBZ and Tax have opposite effects on various signaling pathways. Tax activates both the classical and alternative NF-κB pathways, whereas HBZ specifically suppresses the classical NF-κB pathway by targeting p65. Tax suppresses TGF-β signaling through inhibition of Smad proteins, although HBZ can form a complex with Smad2/3 and p300 to activate the transcription of TGF-β-responsive genes, such as *Foxp3*. Regarding Wnt/β-catenin (canonical Wnt) pathway, Tax activates it by forming complex with DAPLE and DVL, while HBZ suppresses the signaling by interacting with the transcription factors, TCF-1/LEF-1. On the other hand, HBZ up-regulates a noncanonical Wnt ligand, Wnt5a, and supports proliferation and migration of ATL cells, suggesting one of the mechanisms of HBZ-mediated oncogenesis. Recently, we have reported that HBZ suppresses two major apoptotic pathways, Bim-mediated and Fas-mediated pathways, by attenuating the function of FoxO3a. Since Tax is also known to inhibit Fas-mediated apoptosis, it is suggested that HTLV-1-infected cells have redundant means of escaping from apoptosis. We identified other cellular targets of HBZ and Tax by yeast two-hybrid screening and the

transcriptional profiling. We are analyzing their significances in ATL leukemogenesis.

Development of new therapeutic strategies for HTLV-1 infection using the nonhuman primate model: M. MIURA, K. SUGATA, P. MIYAZATO, J. TANABE, J. YASUNAGA, and M. MATSUOKA.

Simian T-cell leukemia virus type 1 (STLV-1) is closely related to HTLV-1. We found that approximately 60% of Japanese macaques are naturally infected with STLV-1, and the dynamics of STLV-1-infected cells in the monkeys are quite similar to that of HTLV-1-infected cells in the human carriers. STLV-1 mainly infects $CD4^+$ T-cells, and induces the clonal proliferation of infected cells. T-cell lymphoma was developed in an STLV-1-infected Japanese macaque. STLV-1 Tax and STLV-1 bZIP factor (SBZ) have the same molecular functions of HTLV-1 Tax and HBZ, respectively; Tax of HTLV-1 and STLV-1 activate NF-κB, CREB, AP-1, NFAT, and Wnt pathways, whereas HBZ and SBZ inhibit them. In addition, HBZ and SBZ activate TGF-β signaling and Tax of both viruses suppress it. A humanized anti-CCR4 monoclonal antibody, mogamulizumab, is now clinically used for the treatment of refractory ATL. Proviral load of STLV-1 in the infected Japanese macaques was drastically decreased by mogamulizumab, suggesting that this antibody has a preventive effect against development of HTLV-1-associated diseases. These observations indicate that STLV-1-infected Japanese macaque is a highly valuable animal model to study the association between viral pathogenesis and immune response in HTLV-1 carriers, and to develop new antiviral treatments. We are trying to establish novel therapeutic strategies using this animal model.

Development of novel small-molecule anti-viral drugs: H. MURAYAMA, K. SHIMURA, and M. MATSUOKA

Current anti-HIV therapy consisting of several classes of anti-HIV drugs potently suppresses HIV-1 replication, and reduction of the viral load to undetectable levels has been sucsesfully achieved. However, HIV-infected individuals need to continue a life-long treatment to prevent the onset of AIDS. Hence, the emergence of drug-resistant viruses is a serious problem. The most effective way to deal with this kind of obstacles is to suppress the drug-resistant variants using anti-HIV drugs with novel modes of action. Until now, we have screened more than 30,000 compounds to identify unique lead anti-HIV drugs. We identified several compounds with anti-HIV activities, and among them, we focused on one small molecle compound, 3,4-dihydro-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine (PD 404182). Although PD 404182 did not show such a strong anti-HIV activity, its mode of action seemed different to pre-existing anti-HIV drugs. From the results of several analyses, we revealed that PD 404182 does not target entry, reverse transcription, or integration, but it inhibits HIV in initial phases of the viral life cycle.

Moreover, PD 404182 preferentially abolishes the infectivity of HIV, but anti-HIV activity is also observed by the pre-treatment of target cells. Interestingly, PD 404182 shows anti-viral activity not only against HIV but also other enveloped viruses, including murine leukemia virus, influenza virus, and herpes simplex virus. Collectively, PD 404182 possesses a broad-spectrum anti-viral activity by affecting factor(s) in the viral envelope.

Comparison of the potency of antiviral agents in cell-free infection versus cell-to-cell transmission of HIV: K. SHIMURA and M. MATSUOKA

HIV infects target cells mainly by two pathways; cell-free infection, in which HIV virions present in the extracellular environment infect target cells, and cell-to-cell transmission, in which HIV is transmitted from infected to uninfected cells through cellular gag or virological synapses. Although it has been reported that the infection efficiency of HIV is higher in cell-to-cell than in cell-free infection pathway, the impact of HIV infection pathways on the potency of anti-HIV drugs is poorly understood. In order to analyse the effect of infection routes on drug susceptibility, we first estabilised an assay system, in which HIV-1 carrying the blue flurescent protein (BFP) gene was employed for precise quantification of HIV-1-infected cells, and a cell-labeling dye was used for the discrimination of donor and target cells. Using this system, we evaluated the anti-HIV-1 activity of several kinds of anti-HIV drugs targeting adsorption, entry, fusion, reverse transcription, and integration steps. We observed weak anti-HIV activity of all tested drugs under the cell-to-cell pathway compared to the cell-free pathway with several-fold ranges. Among the tested agents, HIV integrase inhibitors showed most significant changes. Interestingly, BFP intensity was weak in the presence of integrase inhibitors compared to other classes of anti-HIV drugs under cell-to-cell transmission. It is well known that integrase inhibitors block the integration of proviral DNA into the host genome, and simultaneously, increase the formation of circularized HIV DNA, which is a marker of deficient integration. It has been reported that weak expression of viral genes can be observed from circularized HIV DNA. These observations indicate that, in addition to the transfer of a high copy number of provirus, weak expression of viral product from circularized HIV DNA by integrase inhibitors is another feature of the cell-to-cell HIV infection pathway.

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菅田謙治、安永純一朗、三浦未知、明里宏文、小柳義夫、小原道法、松岡雅雄：組換えウイルスを用いた抗 HTLV-1 ワクチンの作製と Macaque 属での応用、第 61 回日本ウイルス学会学術集会、神戸、2013 年 11 月 10-12 日

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EXPERIMENTAL RESEARCH CENTER FOR INFECTIOUS DISEASES
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Introduction

Our research object is to understand the molecular mechanisms that contribute to epigenetic gene regulation in mammals. To address this issue, we are analyzing biological functions of histone modification and the responsible enzyme, mainly using gene-modifying techniques in mice.

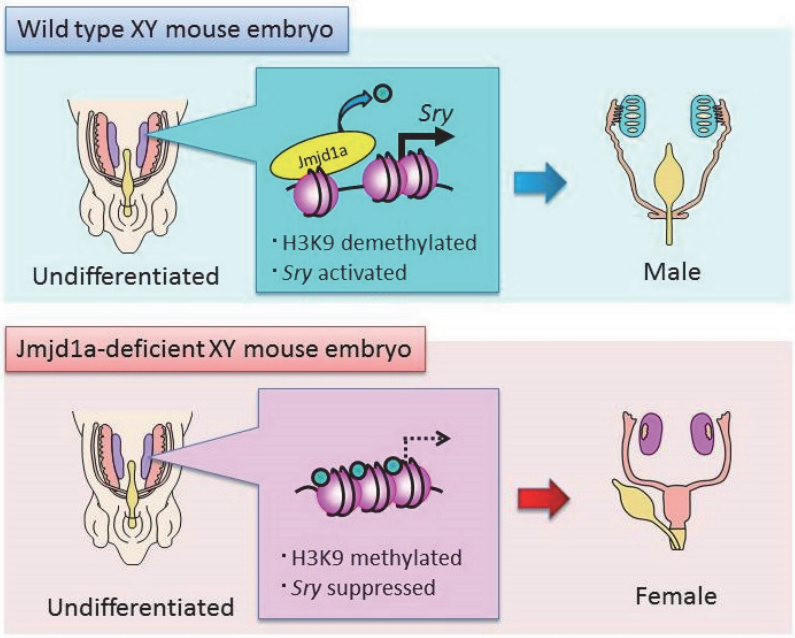
Topics

JMJD1C, a JmjC domain-containing protein, is required for long-term maintenance of male germ cells : S. KUROKI, M. AKIYOSHI and M. TACHIBANA

The JmjC domain-containing proteins are a class of enzymes responsible for histone demethylation. Previous studies revealed that a JmjC domain-containing protein, KDM3A, possesses intrinsic demethylase activity towards lysine 9 of histone H3 and plays essential roles in spermiogenesis. In contrast, the biological roles of JMJD1C, a KDM3A homolog in mice, are largely unknown. Here we present the crucial role of JMJD1C in male gametogenesis. Jmjd1c-deficient males became infertile, due to the progressive reduction of germ cells after 3-months of age. Importantly, Jmjd1c-deficient testes frequently contained abnormal tubules lacking developmentally immature germ cells. The JMJD1C is most abundantly expressed in undifferentiated spermatogonia in mouse testis. The numbers of ZBTB16-positive spermatogonia and apoptotic germ cells in Jmjd1c-deficient testes decreased and increased in aging-dependent manners, respectively. Our studies demonstrated that JMJD1C contributes to the long-term maintenance of the male germ line.

The histone demethylase Jmjd1a controls sex determination by activating Sry expression : S. KUROKI, M. AKIYOSHI and M. TACHIBANA

The mammalian sex determining gene Sry induces differentiation of testes from the bipotential embryonic gonads. Its expression is tightly regulated in a spatio-temporal manner during embryogenesis, and although correct timing of Sry activation is strictly required for its in vivo function, very little is known about the molecules contributing to its regulation. Here we show that the histone H3 lysine 9 (H3K9) demethylase Jmjd1a plays a crucial role in activating Sry expression and therefore controlling sex determination. XY mice deficient for Jmjd1a frequently exhibit male-to-female sex reversal. Interestingly, some of the XY females were fertile. RNA and protein expression analysis demonstrated that loss of Jmjd1a led to a drastic reduction of Sry expression during embryogenesis, strongly suggesting the sex reversal phenotype is due to the perturbation of Sry expression. Accordingly, Jmjd1a protein accumulated at the Sry locus specifically in XY bipotential somatic cells, and loss of Jmjd1a led to a substantial increase of H3K9 methylation and a decrease of H3K4 methylation of the Sry locus in these cells. Our work provides the first evidence for a critical role of histone modification inn mammalian sex determination.



The function of the histone demethylase, Jmjd1a in mouse sex determination.
Jmjd1a contributes to *Sry* expression by removing repressive histone methylation mark from *Sry* locus. XY mice lacking Jmjd1a were frequently sex-reversed due to the insufficient *Sry* expression. H3K9, Histone H3 lysine 9.

List of Publications

Balemans, M.C., Kasri, N.N., Kopanitsa, M.V., Afinowi, N.O., Ramakers, G., Peters, T.A., Beynon, A.J., Janssen, S.M., van Summeren, R.C., Eeftens, J.M., et al. (2013). Hippocampal dysfunction in the Euchromatin histone methyltransferase 1 heterozygous knockout mouse model for Kleefstra syndrome. Human molecular genetics *22*, 852-866.

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総会、つくば、2013 年 5 月

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	Yuriko Nishiyama
	Mai Yoneda
	Daiki Hara

Introduction

We are interested in the pathogenesis of virus infection and wish to contribute to development of better prophylactic and therapeutic interventions against viral diseases. We are conducting research concerning HIV-1 infection, employing non-human primate model, and dengue viruses. Some of the scientific achievements we published in 2012 are briefly summarized below.

Topics

Generation of novel chimeric simian-human immunodeficiency virus (SHIV) strains through intracellular homologous recombination

SHIV, which carries *tat*, *rev*, *vpu* and *env* genes derived from HIV-1 in the backbone of SIV genome, has been a useful virus in the non-human primate model for AIDS. The demand for SHIV strains exhibiting a variety of antigenicity of Env has been increasing further since the identifications of the newer generations of "broadly-neutralizing antibodies" to evaluate their "breadth" of neutralization in context of *in vivo* infection. However, there are only a handful of SHIV strains available in the field. We, who have been generating SHIV strains for more than 20

years, know that generation of a SHIV strain is time-consuming and often ends up with production of a non-infectious virus. We attribute the difficulty to the following two strategies employed for the generation of the virus; 1) utilization of a single infectious molecular clone of HIV-1 as the representative of a given strain, which consists of viral "swarm", and 2) recombination of the HIV-1 genome with that of SIV at restriction sites already present or newly introduced, which are often at the interfaces of the genes. As per the first strategy, it is not known whether a particular molecular clone employed is appropriate as a part of chimeric virus and functional in monkey cells. Concerning the second strategy, there are circulating recombinant forms of HIV-1 strains, naturally occuring chimeric viruses, whose breakpoints are not at the end of certain genes, suggesting that breakpoints based on the restriction sites existing or newly-introduced may not be adequate for a given combination of particular viruses, in this case, SIV and HIV-1. To circumnavigate the above-mentioned uncertainties, we tried to generate SHIV strains through intracellular homologous recombination, a mechanism to repair damage made in double stranded DNA in the cell. We prepared three segments of genes to cover the entire SHIV genome by PCR. For the SIV backbone, we prepared two gene segments from the existing SHIV KS661 plasmid as template. For the HIV-1 gene segment, complementary DNA prepared from the genomic RNA extracted from HIV-1 particles was utilized as template. The three gene segments were co-transfected to human T cell line. To our surprise, recombinant virus emerged in two weeks, compared to months-long efforts of the conventional method for generating recombinant viruses. Employing this method, we generated two strains of novel SHIV, SHIV 97ZA012 and SHIV MNA. SHIV 97ZA012 carries Env derived from HIV-1 97ZA012 strain, a clinical isolate belonging to clade C, a group of viruses responsible for more than 50% of infections worldwide. SHIV MNA carries Env derived from HIV-1 MNA strain, which exhibits neutralization resistant phenotype by conventional neutralization assay but sensitive in the presence of small molecule CD4 mimic. Experimental infection of these SHIVs showed that they were infectious to monkeys. These viruses will be useful to investigate antibody-based control of HIV-1 infection in non-human primate model. We reported our achievements in Virology and Journal of General Virology, respectively.

No viral evolution in the lymph nodes of SIV-infected rhesus macaques during combined antiretroviral therapy

Combined antiretroviral therapy (cART), a combinational administration of three or more anti-HIV-1 drugs, has transformed HIV-1 infection to manageable medical condition. Despite enormous benefit of cART, it is not the ideal therapeutic intervention yet. Even after clinically excellent viral suppression for years, relapse of viremia is commonly observed once the therapy is interrupted, suggesting persistence of virus even during cART. Since a life-long taking of cART causes eventual emergence of resistant mutant virus and adverse effects of the drugs, current goal of

therapeutic intervention against HIV-1 infection is “functional cure”, that is, life-long control of viremia under detection limit without drug administration. To make this materialize, it is necessary to reveal where and how virus persists during cART.

The last year we reported the establishment of long-term cART in SIV-infected rhesus macaques. Despite of clinically successful suppression of viral burdens in the circulation for one year, we detected expression of viral RNA in lymphatic tissues.

We wondered how the viral RNA was expressed. There are two hypotheses concerning maintenance of virus during cART. One is that virus replication persists even during cART in “viral sanctuary”, where anti-viral compounds somehow do not reach. The other is that virus is frozen as provirus integrated in the chromosome of the infected cells prior to the initiation of cART and viral RNA is occasionally expressed from it.

Lentivirus, including HIV-1 and SIV, possesses error-prone reverse transcriptase. It is established that these viruses accumulate mutations with time at a constant rate due to the enzyme. We reasoned that we could tell whether the virus was replicating during the cART we conducted by analyzing accumulation of the mutations in the viral genome. We sequenced *env* gene, which is known to accumulate more mutations than any other viral genes. Genetic analyses revealed that *env* gene expressed in the lymphatic tissues after one year of cART had accumulated little mutations, and was literally indistinguishable to those expressed immediately prior to the initiation of cART. This suggests that there is virtually no virus replication during cART. This implies that the elimination of cells harboring provirus or permanent suppression of viral gene expression from provirus is essential to achieve functional cure. We reported this result in Journal of Virology.

Natural Infection of cynomolgus monkeys with dengue virus occurs in epidemic cycles in the Philippines

Dengue virus, a member of flavivirus, is the causative agent of dengue fever, dengue hemorrhagic fever and dengue shock syndrome. It is an arthropod-borne virus and transmitted to humans by mosquito bites. Besides “epidemic dengue”, which circulates in human population in the tropic regions via mosquitos, there is another cycle of dengue virus infection which is maintained between non-human primate and canopy-dwelling mosquitos, called “sylvatic dengue”. It is believed that epidemic dengue “spilled” from sylvatic cycle by changing tropism to mosquito species in the past. It is known that epidemic and sylvatic dengue viruses are phylogenetically distinct. While there have been a couple of reports concerning sylvatic dengue virus infection in humans, there is only an anecdotal description of “spill back”, infection of non-human primate with epidemic dengue. To gain better understanding on non-human primate in the natural history of dengue virus, we conducted serological and genetic survey of cynomolgus macaques housed in a monkey breeding facility in the Philippines, one of the endemic countries of dengue.

Out of 100 plasma samples of the animals, we detected 35 of them contained antibodies reactive to dengue virus antigen by ELISA. Neutralization test confirmed 8 of 35 samples harbored dengue virus-specific antibodies. Since virus isolation from these positive samples was unsuccessful, we alternatively conducted amplification of viral gene segments by PCR. We successfully amplified a segment of NS1 gene from two samples and E gene from one. Phylogenetic analysis revealed that these genes belonged to epidemic dengue virus, not sylvatic one. The result indicates that non-human primate could serve as the reservoir of epidemic dengue virus. For better surveillance of dengue epidemic, further investigation of non-human primate will be benefitial.

List of Publications

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大附寛幸、五十嵐樹彦、三浦智行：CCR5 指向性サブタイプ C エンベローを持つサル指向性 HIV-1 のブタオザルにおける複製、第 61 回日本ウイルス学会学術交流会、神戸、2013 年 11 月 10-12 日

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大附寛幸、丸田泰広、橋本知恵、鳴海哲夫、廣田雄樹、原田恵嘉、三浦智行、吉村和久、玉村啓和、松下修三、五十嵐樹彦：抗 V3 抗体および低分子 CD4 ミミック曝露後投与によるアカゲザルでの SHIV 複製抑制、第 27 回日本エイズ学会学術集会、熊本、2013 年 11 月 20-22 日

齊藤 暁、大附寛幸、東濃篤徳、鈴木紗織、松田健太、高橋尚史、岩谷靖雅、杉浦 亙、野間口雅子、足立昭夫、保富康宏、俣野哲朗、三浦智行、明里宏文：CCR5 指向性を示す新規サル指向性 HIV-1 はサル個体に持続感染する、第 27 回日本エイズ学会学術集会、熊本、2013 年 11 月 20-22 日

米田 舞、大附寛幸、松下修三、五十嵐樹彦、三浦智行：新規CCR5指向性かつ中和抵抗性SHIV分子クローンの作製及び解析、第27回日本エイズ学会学術集会、熊本、2013年11月20-22日

CENTER FOR EMERGING VIRUS RESEARCH

I. First Group

Members

Assistant Professor (Spe.) Yohei Hizukuri

Introduction

The cell surface of bacteria is exposed to a variety of stresses caused by fluctuations of environmental conditions. The extracytoplasmic stress response (ESR) plays key roles to cope with these cell surface stresses and are important for bacterial survival strategy. ESR is thought to be one of the major systems of virulent bacteria to resist host defense systems triggered by a bacterial infection or invasion. Therefore it is of great importance to understand the whole picture of ESR from a medical viewpoint. This research group focuses on and aims to clarify the mechanism and physiological roles of the σ^E-dependent ESR, one of the major ESR pathways in *Escherichia coli*.

Topics

A structure-based model of substrate-discrimination by a tandem PDZ domain of an *Escherichia coli* intramembrane-cleaving protease RseP: Y. HIZUKURI, T. ODA[1], S. TABATA[2], K. TAMURA-KAWAKAMI[2], R. Oi[1], M. SATO[1], J. TAKAGI[2], T. NOGI[1] and Y. AKIYAMA ([1]Yokohama City Univ., [2]Osaka Univ.)

During the σ^E extracytoplasmic stress response in *Escherichia coli*, cell envelope stresses such as accumulation of malfolded outer membrane proteins (OMPs) or lipopolysaccharides (LPSs) triggers sequential cleavages of RseA, a membrane-spanning anti-σ^E protein that inhibits σ^E activity. The stress cues activate a membrane-anchored protease DegS to cleave RseA, which is followed by the second cleavage by an intramembrane-cleaving protease RseP, leading to eventual activation of σ^E. RseP cleaves RseA only after DegS truncates the periplasmic part of RseA. Our previous works suggested that this two-step proteolysis of RseA is controlled by tandemly-arranged two PDZ domains (PDZ-tandem) in the periplasmic region of RseP.

We determined the crystal structure of the PDZ tandem from an RseP orthologue of a hyperthermophilic bacterium *Aquifex aeolicus* and revealed that the two putative ligand-binding grooves constitute a single pocket-like structure. We built a model of the structure and disposition of the *E. coli* PDZ tandem, which suggest that the PDZ-pocket presumably lies just above the active

center sequestrated within the membrane. Complete removal of the PDZ tandem from *E. coli* RseP led to the deregulated cleavage of RseA independent of prior truncation by DegS *in vivo*. From these and other results we propose that the PDZ tandem serves as a size-exclusion filter to discriminate between the intact and truncated forms of RseA.[1)]

1) Hizukuri, Y., *et al*. (2014) A structure-based model of substrate discrimination by a noncanonical PDZ tandem in the intramembrane-cleaving protease RseP. ***Structure***, 22, 326-336

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Hizukuri, Y., Ito, K., and Akiyama, Y. (2013) RseP Peptidase. Handbook of Proteolytic Enzymes 3rd ed. (ed. Rawlings, N. D. and Salvesen, G.) Elsevier Ltd. pp1546-1550.

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檜作洋平、小田 隆、田畑早苗、川上-田村恵子、佐藤 衛、高木淳一、禾 晃和、秋山芳展：膜内切断プロテアーゼ RseP のタンデム PDZ ドメインが介する切断基質選別機構、第 10 回 21 世紀大腸菌研究会、伊豆、2013 年 6 月 20-21 日

Nogi, T., Hizukuri, Y., Oda, T., Tabata, S., Tamura-Kawakami, K., Sato, M., Takagi, J., and Akiyama, Y.: Structural analysis of the PDZ tandem fragment of the bacterial intramembrane-cleaving protease RseP. International Conference on Structural Genomics 2013, Sapporo, Japan, 29 July - 21 August 2013.

檜作洋平、小田 隆、田畑早苗、川上-田村恵子、佐藤 衛、高木淳一、禾 晃和、秋山芳展：Substrate discrimination mechanism by a PDZ tandem in the intramembrane protease RseP that regulates extracytoplasmic stress response, 第 51 回日本生物物理学会年会、京都、2013 年 10 月 28 日

檜作洋平、小田 隆、田畑早苗、川上-田村恵子、佐藤 衛、高木淳一、禾 晃和、秋山芳展：立体構造解析に基づく大腸菌膜内切断プロテアーゼ RseP のタンデム PDZ ドメインによる切断基質選別機構モデル、第 36 回日本分子生物学会年会、神戸、2013 年 12 月 3 日

II. Second Group

Members

Assistant Professor (Spe.) Akiko Makino

Introduction

Borna disease virus (BDV), which has broad host range in vertebrates, causes persistent infection in nucleus that can lead to neural disorder in horses and sheep. Constitutive activation of NF-κB suppresses the BDV growth, however, NF-κB pathway is not activated in the acute infection of BDV.

Topics

Cross-talk between BDV infection and NF-κB pathway: A. MAKINO, K. FUJINO, K. SOFUKU, S. NAKAMURA, T. HONDA, K. TOMONAGA

Here, to elucidate mechanism for the inhibition of NF-κB activation by BDV infection, we evaluated cross-talk between BDV infection and NF-κB pathway. In THP1-CD14 cells, which has SEAP gene as a NF-κB reporter, BDV infection did not increase SEAP activity from 12 hour to 2 weeks post infection, compared with TLR ligand stimulation. This result is consistent with previous report and suggests BDV has gene(s) that inhibits the activation of NF-κB. To discover common motifs between the amino acid sequences of BDV genes and NF-κB family, we performed Multiple EN for Motif Elicitation analysis and found that nucleoprotein of BDV (BDV-N) and NF-κB1, which is one of the NF-κB family and has a transcription factor activity by processing of precursor p105 form, possess a common ankyrin-like motif.

Pre-treatment with the ankyrin-like peptide of BDV-N suppressed the SEAP activation by the stimulation with TLR ligands, while control peptide had no effect on it. NF-κB1 is phosphorylated by signal transduction and its C-terminal region containing ankyrin repeat undergoes selective degradation by proteasome pathway. Immunoprecipitation assay showed BDV-N was co-precipitated with NF-κB1. Also BDV-N and BDV-N peptide suppressed NF-kB1 degradation by 20S proteasome. These results indicate BDV inhibits NF-κB activation via BDV-N, resulting in its benefit for BDV persistent infection.

List of Publications

Akiko Makino, Kan Fujino, Kozue Sofuku, Shoko Nakamura, Tomoyuki Honda, Keizo Tomonaga.: Inhibition of NF-κB activation by peptide derived from nucleoprotein of Borna disease virus. 15th International Negative Strand Virus Meeting, Granada, Spain, 16-21 June 2013.

牧野晶子、藤野 寛、惣福 梢、中村祥子、伊藤睦美、本田知之、新矢恭子、河岡義裕、朝長啓造：ウイルスタンパク質内の TLR シグナル伝達阻害ペプチドの探索と評価、第六回ボルナウイルス研究会、東京、2013 年 3 月 14 日

牧野晶子、藤野 寛、惣福 梢、中村祥子、本田知之、朝長啓造：ボルナ病ウイルス N タンパク質のアンキリン様配列を介した NF-κ B 活性化阻害、第 61 回日本ウイルス学会、神戸、2013 年 11 月 10-12 日

REPRODUCTIVE ENGINEERING TEAM

Members

Technical Specialist	**Hitoshi Miyachi**
Technical Staff	**Satsuki Kitano (Konaka)**

Introduction

Reproductive engineering team is a support unit for generating transgenic mouse (Tg) and knockout mouse (KO) under the animal committee of our institute. We also perform cryopreservation of mouse fertilized eggs. Current staffs are Kitano and Miyachi. Results of last three years are as follows.

1) Freezing embryos

2011	117 strains	25,130 embryos
2012	140 strains	32,836 embryos
2013	198 strains	52,272 embryos

2) Transgenic mouse production with cloned DNAs

	No of constructs	No of embryos injected	No of transgenic pups obtained
2011	81	29,031	227(0.8%)
2012	77	31,452	176(0.6%)
2013	71	27,924	78(0.3%)

3) Production of chimeric mouse

	No of ES clones	No of embryos injected	No of coatcolor chimera obtained
2011	107	5,828	324(5.5%)
2012	63	5,145	192(3.7%)
2013	70	5,510	129(2.3%)

List of Publications

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Kuroki, S., Akiyoshi, M., Tokura, M., Miyachi, H., Nakai, Y., Kimura, H., Shinkai, Y., and Tachibana, M. (2013). JMJD1C, a JmjC Domain-Containing Protein, Is Required for Long-Term Maintenance of Male Germ Cells in Mice. Biol. Reprod. 89, 93.

Kuroki, S., Matoba, S., Akiyoshi, M., Matsumura, Y., Miyachi, H., Mise, N., Abe, K., Ogura, A., Wilhelm, D., Koopman, P., et al. (2013). Epigenetic regulation of mouse sex determination by the histone demethylase Jmjd1a. Science *341*, 1106-1109.

Tani-Ichi, S., Shimba, A., Wagatsuma, K., Miyachi, H., Kitano, S., Imai, K., Hara, T., and Ikuta, K. (2013). Interleukin-7 receptor controls development and maturation of late stages of thymocyte subpopulations. Proc. Natl. Acad. Sci. U. S. A. *110*, 612-617.

COMPUTER NETWORK OF INSTITUTE FOR VIRUS RESEARCH

Assistant Professor　　Keiko Takemoto

Introduction

Institute for Virus Research LAN system (IVR-LAN) has administrated by the network committee consisted of four staffs (Prof. Toyoshima, Prof. Akiyama, Associate Prof. Mori and Instructor Takemoto). IVR-LAN service has covered for researchers of some medical departments as well as IVR, and the primary purpose of IVR-LAN is to offer accessibility to the Internet in support of their studies. IVR-LAN has provided a variety of network services, including E-Mail, WEB-mail, WWW, File-sharing, online reservation of seminar rooms, SSH and all Outgoing TCP services except for P2P. Main services are working on Sun Sparc platform with Solaris 10 and DELL with Linux.

This year we installed plone server which enabled us to maintain our local web site by other staffs and allowed our institute members to upload documents on a board portal and hold paperless meeting with tablet devices.

However IVR-LAN has adequately equipped, we must have a responsibility for sending/getting data. A few accidents have occurred in this year. IVR-LAN users need to get certifications of training of e-learning course which is provided by Institute for Information Management and Communication of Kyoto university.

In addition to the administration of network, Takemoto have studied the epigenetic regulation of mouse endogenous retroviruses during cell differentiation. Conditional knockdown mice of histone methyltransferase ESET, and/or DNA de novo methyltransferase Dnmt1 have been analyzed with RNA-seq and ChIP-seq.

List of Publications

竹本経緯子：H3K9 メチル化による内在性レトロウイルスの制御、NGS 現場の会第 3 回研究会、神戸、2013 年 9 月 4-5 日

井上真悠子、黒田貴雄、加藤雅紀、竹本経緯子、駒井 妙、眞貝洋一、水谷健一：Prdm8 は発生期大脳皮質の多極性形態期を調節する、第 36 回日本分子生物学会年会、神戸、2013 年 12 月 3-6 日